
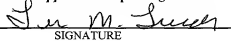


FORM PTO-1390 U.S. DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE (MODIFIED)		ATTORNEY'S DOCKET NUMBER X-11260	
TRANSMITTAL LETTER TO THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US) CONCERNING A FILING UNDER 35 U.S.C. 371		U.S. APPLICATION NO. (if known, see 37 C.F.R. 1.5) 10/018214	
INTERNATIONAL APPLICATION NO. PCT/US00/15017	INTERNATIONAL FILING DATE 08 June 2000 (08.06.00)	PRIORITY DATE CLAIMED 15 July 1999 (15.07.99)	
TITLE OF INVENTION: PSEUDOMYCIN N-ACYL SIDE-CHAIN ANALOGS			
APPLICANT(S) FOR DO/EO/US: Matthew David Belvo, Shu Hui Chen, Christopher William Doecke, Sarah Lynne Hellman, James Andrew Jamison, Lawrence Edward Patterson, Michael John Rodriguez, Xicheng David Sun, William Wilson Turner, Venkatraghavan Vasudevan, and Mark James Zweifel			
Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:			
1.	<input checked="" type="checkbox"/>	This is a FIRST submission of items concerning a filing under 35 U.S.C. 371.	
2.	<input type="checkbox"/>	This is a SECOND or SUBSEQUENT submission of items concerning a filing under 35 U.S.C. 371.	
3.	<input type="checkbox"/>	This express request to begin national examination procedures (35 U.S.C. 371(f)) at any time rather than delay examination until the expiration of the applicable time limit set in 35 U.S.C. 371(b) and PCT Articles 22 and 39(1).	
4.	<input checked="" type="checkbox"/>	A proper Demand for International Preliminary Examination was made by the 19th month from the earliest claimed priority date.	
5.	<input checked="" type="checkbox"/>	A copy of the International Application as filed (35 U.S.C. 371(c)(2))	
	<input type="checkbox"/>	a. is transmitted herewith (required only if not transmitted by the International Bureau).	
	<input type="checkbox"/>	b. has been transmitted by the International Bureau.	
	<input checked="" type="checkbox"/>	c. is not required, as the application was filed in the United States Receiving Office (RO/US).	
6.	<input type="checkbox"/>	A translation of the International Application into English (35 U.S.C. 371(c)(2)).	
7.	<input checked="" type="checkbox"/>	Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. 371(c)(3))	
	<input type="checkbox"/>	a. are transmitted herewith (required only if not transmitted by the International Bureau).	
	<input type="checkbox"/>	b. have been transmitted by the International Bureau.	
	<input type="checkbox"/>	c. have not been made; however, the time limit for making such amendments has NOT expired.	
	<input checked="" type="checkbox"/>	d. have not been made and will not be made.	
8.	<input type="checkbox"/>	A translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371(c)(3)).	
9.	<input checked="" type="checkbox"/>	An oath or declaration of the inventor(s) (35 U.S.C. 371(c)(4)).	
10.	<input checked="" type="checkbox"/>	A copy of the International Preliminary Examination Report (IPER), including any annexes, and, if not in English, an English language translation of the annexes to the IPER under PCT Article 36 (35 U.S.C. 371(c)(5)).	
Items 11. to 16. below concern document(s) or information included:			
11.	<input type="checkbox"/>	An Information Disclosure Statement under 37 CFR 1.97 and 1.98.	
12.	<input checked="" type="checkbox"/>	An assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included.	
13.	<input checked="" type="checkbox"/>	A FIRST preliminary amendment.	
	<input type="checkbox"/>	A SECOND or SUBSEQUENT preliminary amendment.	
14.	<input type="checkbox"/>	A substitute specification.	
	<input type="checkbox"/>	A change of power of attorney and/or address letter.	
15.	<input type="checkbox"/>	Other items or information:	

JCO7 Rec'd PCT/PTO 1 3 DEC 2001

U.S. APPLICATION NO. (if known, see 37 C.F.R. 1.5)		INTERNATIONAL APPLICATION NO.		ATTORNEY'S DOCKET NUMBER	
10/018214		PCT/US00/15017		X-11260	
17.	<input checked="" type="checkbox"/>	The following fees are submitted:			CALCULATIONS PTO USE ONLY
BASIC NATIONAL FEE (37 CFR 1.492(a)(1)-(5)): Neither international preliminary examination fee (37 CFR 1.482) nor international search fee (37 CFR 1.445(a)(2)) paid to USPTO and International Search Report not prepared by the EPO or JPO. \$1040.00					
International preliminary examination fee (37 CFR 1.482) not paid to USPTO but International Search Report prepared by the EPO or JPO \$890.00					
International preliminary examination fee (37 CFR 1.482) not paid to USPTO but international search fee (37 CFR 1.445(a)(2)) paid to USPTO..... \$740.00					
International preliminary examination fee paid to USPTO (37 CFR 1.482) but all claims did not satisfy provisions of PCT Article 33(1)-(4) \$710.00					
International preliminary examination fee paid to USPTO (37 CFR 1.482) and all claims satisfied provisions of PCT Article 33(2)-(4) \$100.00					
ENTER APPROPRIATE BASIC FEE AMOUNT =					\$ 890.00
Surcharge of \$130.00 for furnishing the oath or declaration later than <u>20</u> <u>30</u> months from the earliest claimed priority date (37 CFR 1.492(e)).					\$
CLAIMS	NUMBER FILED	NUMBER EXTRA	RATE		
Total claims	15 -20=	0	X \$18.00	\$	
Independent claims	4 - 3=	1	X \$84.00	\$	84.00
MULTIPLE DEPENDENT CLAIM(S) (if applicable)			+ \$280.00	\$	
TOTAL OF ABOVE CALCULATIONS =					\$ 84.00
Reduction by 1/2 for filing by small entity, if applicable. Verified Small Entity Statement must also be filed (Note 37 CFR 1.9, 1.27, 1.28).					\$
SUBTOTAL =					\$ 974.00
Processing fee of \$130.00 for furnishing English translation later than <u>20</u> <u>30</u> months from the earliest claimed priority date (37 CFR 1.492(f)).					\$
TOTAL NATIONAL FEE =					\$ 974.00
Fee for recording the enclosed assignment (37 CFR 1.21(h)). The assignment must be accompanied by an appropriate cover sheet (37 CFR 3.28, 3.31).					\$
\$40.00 per property					
TOTAL FEES ENCLOSED =					\$ 974.00
					Amount to be refunded
					charged
a.	<input type="checkbox"/>	A check in the amount of \$_____ to cover the above fees is enclosed.			
b.	<input checked="" type="checkbox"/>	Please charge my Deposit Account No. 05-0840 in the amount of \$974.00 to cover the above fees. A duplicate copy of this sheet is enclosed.			
c.	<input checked="" type="checkbox"/>	The Commissioner is hereby authorized to charge any additional fees which may be required, or credit any overpayment to Deposit Account No. 05-0840. A duplicate copy of this sheet is enclosed.			
NOTE: Where an appropriate time limit under 37 CFR 1.494 or 1.495 has not been met, a petition to revive (37 CFR 1.137(a) or (b)) must be filed and granted to restore the application to pending status.					
SEND ALL CORRESPONDENCE TO: ELI LILLY AND COMPANY PATENT DIVISION/1104 LILLY CORPORATE CENTER INDIANAPOLIS, INDIANA 46285					
		 25885 PATENT TRADEMARK OFFICE		SIGNATURE  Tina M. Tucker NAME	
13 Dec 2001 Date		47.145 REGISTRATION NUMBER		(317) 277-3537 TELEPHONE NUMBER	

10/018214

JG07 Rec'd PCT/PTO 13 DEC 2001

"Express Mail" mailing label number EL 230530064 USDate of Deposit Dec. 13, 2001

I hereby certify that this paper or fee is being deposited with the United States Postal Service "Express Mail Post Office to Addressee" service under 37 C.F.R. 1.10 on the date indicated above and is addressed to the Assistant Commissioner for Patents, Washington, D.C. 20231.

Queen Thomas
 Printed Name

Queen Thomas
 Signature

PATENT APPLICATION
IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant(s):	Belvo, et al.)
)
Serial No.:	Unknown)
) Group Art Unit:
Filed:	June 8, 2000) Unknown
)
For:	Pseudomycin N-Acyl Side-Chain) Examiner:
	Analogs) Unknown
)
Docket No.:	X-11260)

Preliminary Amendment

Assistant Commissioner for Patents
 Washington, D. C. 20231

Sir:

Applicants submit the following preliminary amendments and remarks in connection with the filing of the above-identified application.

Please amend the application as follows:

In the Specification

On page 4 of the specification, after the sentence, "R^f is hydrogen, hydroxy, C₁-C₄ alkoxy...;" please insert the following

--R^d is hydrogen;--

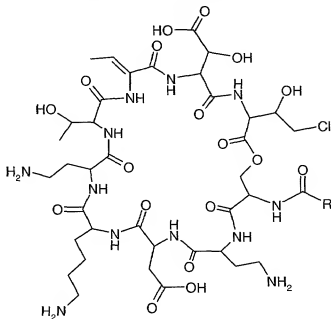
On page 6 of the specification, line 8, please replace "an antifungal" with "a fungal".

In the Claims

Please cancel Claim 5 without prejudice or disclaimer of any of the subject matter contained herein.

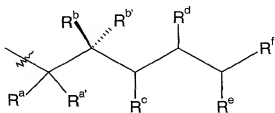
Please amend the claims as follows:

- (Amended) A compound represented by structure I



I

wherein R is



where

R^a and R^{a'} are independently hydrogen or methyl, or either R^a or R^{a'} is alkyl amino, taken together with R^b or R^{b'} forms a six-membered cycloalkyl ring, a six-membered aromatic ring or a double bond, or taken together with R^c forms a six-membered aromatic ring;

R^b and R^{b'} are independently hydrogen, halogen, or methyl, or either R^b or R^{b'} is amino, alkylamino, α -acetoacetate, methoxy, or hydroxy provided that R^{b'} is not

hydroxy when R^a, R^b, R^d, R^e are hydrogen, R^c is hydrogen and R^f is *n*-hexyl, *n*-octyl or *n*-decyl, or R^a, R^b, R^d, R^e are hydrogen, R^c is hydroxy and R^f is *n*-octyl, *n*-nonyl, or *n*-decyl;

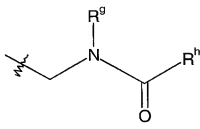
R^c is hydrogen, hydroxy, C_1 - C_4 alkoxy, hydroxyalkoxy, or taken together with R^e forms a 6-membered aromatic ring or C_5 - C_6 cycloalkyl ring;

R^d is hydrogen;

R^e is hydrogen, or taken together with R^f is a six-membered aromatic ring, C_5 - C_{14} alkoxy substituted six-membered aromatic ring, or C_5 - C_{14} alkyl substituted six-membered aromatic ring, and

R^f is C_8 - C_{18} alkyl, C_5 - C_{11} alkoxy, or biphenyl; or

R is

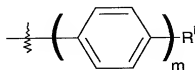


where

R^g is hydrogen, or C_1 - C_{13} alkyl, and

R^h is C_1 - C_{15} alkyl, C_4 - C_{15} alkoxy, $(C_1$ - C_{10} alkyl)phenyl, $-(CH_2)_n$ -aryl, or $-(CH_2)_n$ -(C_5 - C_6 cycloalkyl), where $n = 1-2$; or

R is

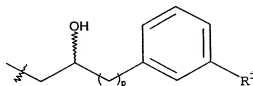


where

R^i is a hydrogen, halogen, or C_5 - C_8 alkoxy, and

m is 1, 2 or 3;

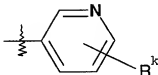
R is



where

R^j is C_5 - C_{14} alkoxy or C_5 - C_{14} alkyl, and $p = 0, 1$ or 2 ;

R is



where

R^k is C_5 - C_{14} alkoxy; or

R is $-(CH_2)_n-NR^m-(C_{13}-C_{18} \text{ alkyl})$, where R^m is H, $-CH_3$ or

$-C(O)CH_3$; and

pharmaceutically acceptable salts and solvates thereof.

6. (Amended) A pharmaceutical formulation comprising said pseudomycin compound or said pharmaceutically acceptable salt or solvate thereof of Claim 2 and a pharmaceutically acceptable carrier, diluent, buffer, or excipient.

7. (Amended) A method for treating a fungal infection in an animal in need thereof, comprising the steps of administering to said animal said pseudomycin compound or said pharmaceutically acceptable salt or solvate thereof of Claim 2.

Please add the following new claims:

--16. A pharmaceutical formulation comprising said pseudomycin compound or said pharmaceutically acceptable salt or solvate thereof of Claim 1 and a pharmaceutically acceptable carrier, diluent, buffer, or excipient.--

--17. A method for treating a fungal infection in an animal in need thereof, comprising the steps of administering to said animal said pseudomycin compound or said pharmaceutically acceptable salt or solvate thereof of Claim 1.--

REMARKS

Claim 5 has been canceled. Claims 1 and 6-7 have been amended. Claims 16 and 17 have been newly added. Basis for this new claims can be found on page 6, lines 3-10. Thus, claims 1-4 and 6-17 are presently in the application.

In regard to claim 1, the variable " R^d " was included in the definition of " R ", but the definition of R^d , itself, was inadvertently omitted. However, support for amending claim 1 to include a definition of R^d can be found in the definition of " R^c " in claim 1 as originally filed. Inasmuch as R^c and R^e may form a six-membered aromatic ring, R^d , thus, must at least be hydrogen. As such, R^d in claim 1 and the specification at page 4 has been amended to recite hydrogen.

In regard to claims 6-7, these claims have been amended to include a pharmaceutically acceptable salt or solvate thereof, a buffer, a diluent or a excipient in the formulation. Basis for the amendments can be found in claim 1 and on page 24, lines 18-25, to page 25, lines 1-4. Additionally, claims 6-7 have been amended to correct the antecedent basis (" a " has been replaced by "said"). Furthermore, claim 7 has been amended to correct an obvious typographical error ("an antifungal" infection has been replaced by "a fungal" infection). Basis for this amendment can be found on page 26, lines 5-23.

Attached hereto is a marked-up version of the changes made to the specification and claims by the current amendment. The attached page is captioned "**Version with markings to show changes made.**"

For the Examiner's convenience, a clean claim set is attached.

Early and favorable action on the merits is respectfully requested.

Please charge any fees or credit any overpayment in connection with this application which may be required by this or any related paper to Deposit Account No. 05-0840.

If the Examiner has any questions, or would like to discuss any matters in connection with this application, he or she is invited to contact the undersigned at (317) 277-3537.

Respectfully submitted,

Tina M. Tucker

Tina M. Tucker
Agent for Applicants
Registration No. 47,145
Phone: 317-277-3537

Eli Lilly and Company
Patent Division/TMT
Lilly Corporate Center
Indianapolis, Indiana 46285

16 Nov 2001

Attachments: Clean Claim Set

VERSION WITH MARKINGS TO SHOW CHANGES MADE

In the Specification

On page 4 of the specification, after the sentence, “R^c is hydrogen, hydroxy, C₁-C₄ alkoxy...;” please insert the following

--R^d is hydrogen;--

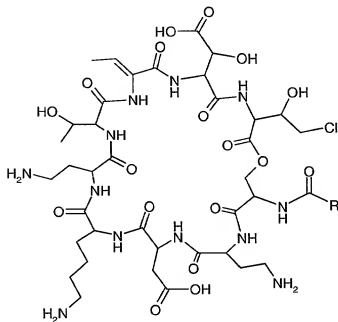
On page 6 of the specification, line 8, please replace “an antifungal” with “a fungal”.

In the claims:

Claim 5 has been cancelled.

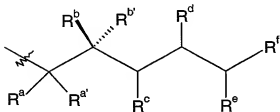
The claims have been amended as follows:

1. (Amended) A compound represented by structure I



I

wherein R is



where

R^a and $R^{a'}$ are independently hydrogen or methyl, or either R^a or $R^{a'}$ is alkyl amino, taken together with R^b or $R^{b'}$ forms a six-membered cycloalkyl ring, a six-membered aromatic ring or a double bond, or taken together with R^c forms a six-membered aromatic ring;

R^b and $R^{b'}$ are independently hydrogen, halogen, or methyl, or either R^b or $R^{b'}$ is amino, alkylamino, α -acetoacetate, methoxy, or hydroxy provided that $R^{b'}$ is not hydroxy when R^a , R^b , R^d , R^e are hydrogen, R^c is hydrogen and R^f is *n*-hexyl, *n*-octyl or *n*-decyl, or R^a , R^b , R^d , R^e are hydrogen, R^c is hydroxy and R^f is *n*-octyl, *n*-nonyl, or *n*-decyl;

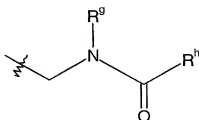
R^c is hydrogen, hydroxy, C_1 - C_4 alkoxy, hydroxyalkoxy, or taken together with R^e forms a 6-membered aromatic ring or C_5 - C_6 cycloalkyl ring;

R^d is hydrogen;

R^e is hydrogen, or taken together with R^f is a six-membered aromatic ring, C_5 - C_{14} alkoxy substituted six-membered aromatic ring, or C_5 - C_{14} alkyl substituted six-membered aromatic ring, and

R^f is C_8 - C_{18} alkyl, C_5 - C_{11} alkoxy, or biphenyl; or

R is

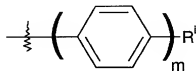


where

R^g is hydrogen, or C_1 - C_{13} alkyl, and

R^h is C_1 - C_{15} alkyl, C_4 - C_{15} alkoxy, $(C_1$ - C_{10} alkyl)phenyl, $-(CH_2)_n$ -aryl, or $-(CH_2)_n$ - $(C_5$ - C_6 cycloalkyl), where $n = 1-2$; or

R is

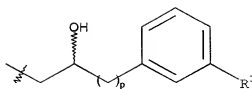


where

R^i is a hydrogen, halogen, or C_5 - C_8 alkoxy, and

m is 1, 2 or 3;

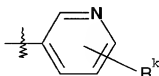
R is



where

R^j is C₅-C₁₄ alkoxy or C₅-C₁₄ alkyl, and $p = 0, 1$ or 2 ;

R is



where

R^k is C₅-C₁₄ alkoxy; or

R is $-(CH_2)-NR^m-(C_{13}-C_{18} \text{ alkyl})$, where R^m is H, $-CH_3$ or

$-C(O)CH_3$; and

pharmaceutically acceptable salts and solvates thereof.

6. (Amended) A pharmaceutical formulation comprising [a] said pseudomycin compound or said pharmaceutically acceptable salt or solvate thereof of Claim 2 and a pharmaceutically acceptable carrier, diluent, buffer, or excipient.

7. (Amended) A method for treating [an antifungal] a fungal infection in an animal in need thereof, comprising the steps of administering to said animal [a] said pseudomycin compound [of Claim 2] or said pharmaceutically acceptable salt or solvate thereof of Claim 2.

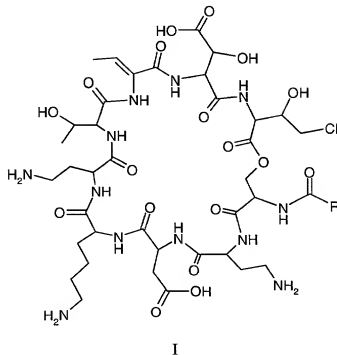
Claims 16 and 17 have been added as follows:

--16. A pharmaceutical formulation comprising said pseudomycin compound or said pharmaceutically acceptable salt or solvate thereof of Claim 1 and a pharmaceutically acceptable carrier, diluent, buffer, or excipient.--

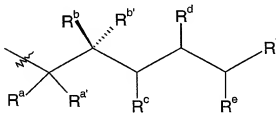
--17. A method for treating a fungal infection in an animal in need thereof, comprising the steps of administering to said animal said pseudomycin compound or said pharmaceutically acceptable salt or solvate thereof of Claim 1.--

CLEAN CLAIM SET

1. A compound represented by structure I



wherein R is



where

R^a and $R^{a'}$ are independently hydrogen or methyl, or either R^a or $R^{a'}$ is alkyl amino, taken together with R^b or $R^{b'}$ forms a six-membered cycloalkyl ring, a six-membered aromatic ring or a double bond, or taken together with R^c forms a six-membered aromatic ring;

R^b and $R^{b'}$ are independently hydrogen, halogen, or methyl, or either R^b or $R^{b'}$ is amino, alkylamino, α -acetoacetate, methoxy, or hydroxy provided that $R^{b'}$ is not hydroxy when R^a , R^b , R^d , R^e are hydrogen, R^c is hydrogen and R^f is *n*-hexyl, *n*-octyl or *n*-decyl, or R^a , R^b , R^d , R^e are hydrogen, R^c is hydroxy and R^f is *n*-octyl, *n*-nonyl, or *n*-decyl;

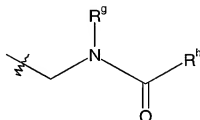
R^c is hydrogen, hydroxy, C₁-C₄ alkoxy, hydroxyalkoxy, or taken together with R^e forms a 6-membered aromatic ring or C₅-C₆ cycloalkyl ring;

R^d is hydrogen;

R^e is hydrogen, or taken together with R^f is a six-membered aromatic ring, C_5 - C_{14} alkoxy substituted six-membered aromatic ring, or C_5 - C_{14} alkyl substituted six-membered aromatic ring, and

R^f is C_8 - C_{18} alkyl, C_5 - C_{11} alkoxy, or biphenyl; or

R is

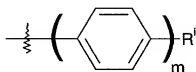


where

R^g is hydrogen, or C_1 - C_{13} alkyl, and

R^h is C_1 - C_{15} alkyl, C_4 - C_{15} alkoxy, $(C_1$ - C_{10} alkyl)phenyl, $-(CH_2)_n$ -aryl, or $-(CH_2)_n$ - $(C_5$ - C_6 cycloalkyl), where $n = 1-2$; or

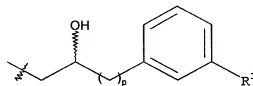
R is



where

R^i is a hydrogen, halogen, or C_5 - C_8 alkoxy, and
 m is 1, 2 or 3;

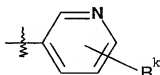
R is



where

R^j is C_5 - C_{14} alkoxy or C_5 - C_{14} alkyl, and $p = 0, 1$ or 2 ;

R is



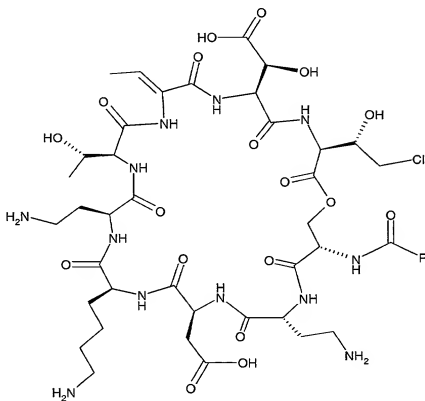
where

R^k is C_5 - C_{14} alkoxy; or

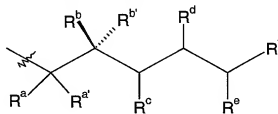
R is $-(CH_2)-NR^m-(C_{13}-C_{18} \text{ alkyl})$, where R^m is H, $-CH_3$ or $-C(O)CH_3$; and

pharmaceutically acceptable salts and solvates thereof.

2. The compound of Claim 1 wherein structure I has the following stereochemistry



3. The compound of Claim 1 wherein R is



where

R^a and $R^{a'}$ are independently hydrogen or methyl, or either R^a or $R^{a'}$ is alkyl amino, taken together with R^b or $R^{b'}$ forms a six-membered cycloalkyl ring, a six-

membered aromatic ring or a double bond, or taken together with R^c forms a six-membered aromatic ring;

R^b and R^{b'} are independently hydrogen, halogen, or methyl, or either R^b or R^{b'} is amino, alkylamino, α -acetoacetate, methoxy, or hydroxy provided that R^{b'} is not hydroxy when R^a, R^b, R^d, R^e are hydrogen, R^c is hydrogen and R^f is *n*-hexyl, *n*-octyl or *n*-decyl, or R^a, R^b, R^d, R^e are hydrogen, R^c is hydroxy and R^f is *n*-octyl, *n*-nonyl, or *n*-decyl;

R^e is hydrogen, hydroxy, C₁-C₄ alkoxy, hydroxyalkoxy, or taken together with R^e forms a 6-membered aromatic ring or C₅-C₆ cycloalkyl ring;

R^e is hydrogen, or taken together with R^f is a six-membered aromatic ring, C₅-C₁₄ alkoxy substituted six-membered aromatic ring, or C₅-C₁₄ alkyl substituted six-membered aromatic ring, and

R^f is C₈-C₁₈ alkyl, C₅-C₁₁ alkoxy, or biphenyl.

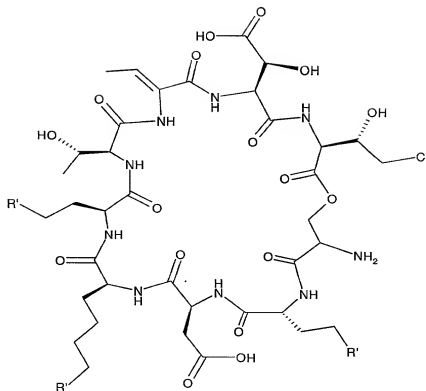
4. The compound of Claim 3 wherein R^{b'} is hydroxy provided that R^c is not hydrogen when R^a, R^b, R^d, R^e are hydrogen and R^f is *n*-hexyl, *n*-octyl or *n*-decyl, or R^c is not hydroxy when R^f is *n*-octyl, *n*-nonyl, or *n*-decyl.

6. A pharmaceutical formulation comprising said pseudomycin compound or said pharmaceutically acceptable salt or solvate thereof of Claim 2 and a pharmaceutically acceptable carrier, diluent, buffer, or excipient.

7. A method for treating a fungal infection in an animal in need thereof, comprising the steps of administering to said animal said pseudomycin compound or said pharmaceutically acceptable salt or solvate thereof of Claim 2.

8. A process for producing a pseudomycin nucleus comprising the steps of providing a pseudomycin compound having an N-acyl alkyl side-chain containing at least one gamma or delta hydroxy group and reacting said pseudomycin compound with an acid to produce said pseudomycin nucleus.

9. The process of Claim 8 wherein said pseudomycin nucleus is represented by structure I-A



I-A

wherein R' is -NH₂ or -NHp-Pg where Pg is an amino protecting group and p is 0 or 1.

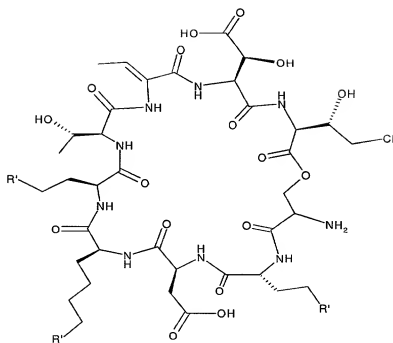
10. The process of Claim 8 wherein said pseudomycin compound having an N-acyl alkyl side-chain containing at least one gamma or delta hydroxy group is selected from the group consisting of pseudomycin A, pseudomycin A' and pseudomycin C.

11. The process of Claim 8 wherein said acid is trifluoroacetic acid or acetic acid.

12. The process of Claim 11 wherein said acid is trifluoroacetic acid.

13. A pseudomycin nucleus prepared by the process of Claim 8.

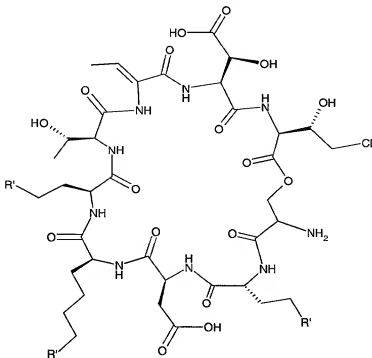
14. The pseudomycin nucleus of Claim 13 wherein said nucleus is represented by structure I-A



I-A

wherein R' is -NH₂ or -NHp-Pg where Pg is an amino protecting group and p is 0 or 1.

15. A pseudomycin nucleus represented by structure I-A



I-A

wherein R' is -NH₂ or -NHp-Pg where Pg is an amino protecting group and p is 0 or 1.

16. A pharmaceutical formulation comprising said pseudomycin compound or said pharmaceutically acceptable salt or solvate thereof of Claim 1 and a pharmaceutically acceptable carrier, diluent, buffer, or excipient.

17. A method for treating a fungal infection in an animal in need thereof, comprising the steps of administering to said animal said pseudomycin compound or said pharmaceutically acceptable salt or solvate thereof of Claim 1.

PSEUDOMYCIN N-ACYL SIDE-CHAIN ANALOGS

5

FIELD OF THE INVENTION

The present invention relates to pseudomycin compounds, in particular, semi-synthetic pseudomycin compounds having novel N-acyl side-chains.

10

BACKGROUND OF THE INVENTION

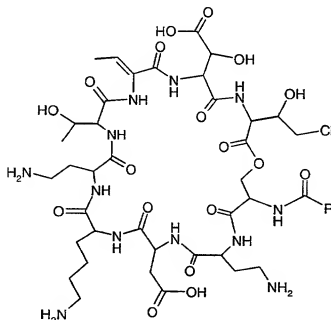
Pseudomycins are natural products isolated from liquid cultures of *Pseudomonas syringae* (plant-associated bacterium) and have been shown to have antifungal activities. (see i.e., Harrison, L., et al., "Pseudomycins, a family of novel peptides from *Pseudomonas syringae* possessing broad-spectrum antifungal activity," J. Gen. Microbiology, **137**(12), 2857-65 (1991) and US Patent Nos. 5,576,298 and 5,837,685) Unlike the previously described antimycotics from *P. syringae* (e.g., syringomycins, syringotoxins and syringostatins), pseudomycins A-C contain hydroxyaspartic acid, aspartic acid, serine, dehydroaminobutyric acid, lysine and diaminobutyric acid. The peptide moiety for pseudomycins A, A', B, B', C, C' corresponds to L-Ser-D-Dab-L-Asp-L-Lys-L-Dab-L-aThr-Z-Dhb-L-Asp(3-OH)-L-Thr(4-Cl) with the terminal carboxyl group closing a macrocyclic ring on the OH group of the N-terminal

- Ser. The analogs are distinguished by the N-acyl side chain, i.e., pseudomycin A is N-acylated by 3,4-dihydroxytetradecanoyl, pseudomycin A' by 3,4-dihydroxypentadecanoyl, pseudomycin B by 3-hydroxytetradecanoyl, pseudomycin B' by 3-hydroxydodecanoyl, pseudomycin C by 3,4-dihydroxyhexadecanoyl and pseudomycin C' by 3-hydroxyhexadecanoyl. (see i.e., Ballio, A., et al., "Novel bioactive lipodepsipeptides from *Pseudomonas syringae*: the pseudomycins," FEBS Letters, **355**(1), 96-100, (1994) and Coiro, V.M., et al., "Solution conformation of the *Pseudomonas syringae* MSU 16H phytotoxic lipodepsipeptide Pseudomycin A determined by computer simulations using distance geometry and molecular dynamics from NMR data," Eur. J. Biochem., **257**(2), 449-456 (1998).)

Pseudomycins are known to have certain adverse biological effects. For example, destruction of the endothelium of the vein, destruction of tissue, inflammation, and local toxicity to host tissues have been observed when pseudomycin is administered intravenously. Therefore, there is a need to identify compounds within this class that are useful for treating fungal infections without the currently observed adverse side effects.

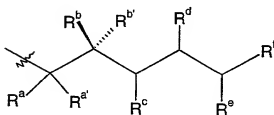
BRIEF SUMMARY OF THE INVENTION

The present invention provides pseudomycin compounds represented by the following structure which are useful as antifungal agents or in the design of antifungal agents.



I

wherein R is



where

- 10 R^a and $R^{a'}$ are independently hydrogen or methyl, or either R^a or $R^{a'}$ is alkyl amino, taken together with R^b or $R^{b'}$ forms a six-membered cycloalkyl ring, a six-

membered aromatic ring or a double bond, or taken together with R^c forms a six-membered aromatic ring;

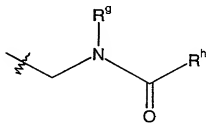
R^b and R^{b'} are independently hydrogen, halogen, or methyl, or either R^b or R^{b'} is amino, alkylamino, α-acetoacetate, methoxy, or hydroxy provided that R^{b'} is not hydroxy when R^a, R^b, R^d, R^e are hydrogen, R^c is hydrogen and R^f is *n*-hexyl, *n*-octyl or *n*-decyl, or R^a, R^b, R^d, R^e are hydrogen, R^c is hydroxy and R^f is *n*-octyl, *n*-nonyl, or *n*-decyl;

R^c is hydrogen, hydroxy, C₁-C₄ alkoxy, hydroxyalkoxy, or taken together with R^e forms a 6-membered aromatic ring or C₅-C₆ cycloalkyl ring;

R^e is hydrogen, or taken together with R^f is a six-membered aromatic ring, C₅-C₁₄ alkoxy substituted six-membered aromatic ring, or C₅-C₁₄ alkyl substituted six-membered aromatic ring, and

R^f is C₈-C₁₈ alkyl, C₅-C₁₁ alkoxy, or biphenyl; or

R is

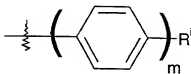


where

R^g is hydrogen, or C₁-C₁₃ alkyl, and

R^h is C_1 - C_{15} alkyl, C_4 - C_{15} alkoxy, (C_1 - C_{10} alkyl)phenyl, $-(CH_2)_n$ -aryl, or $-(CH_2)_n$ -(C_5 - C_6 cycloalkyl), where $n = 1$ or 2 ; or

R is

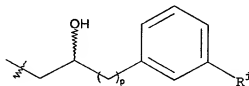


5

where

R^i is a hydrogen, halogen, or C_5 - C_8 alkoxy, and m is 1, 2 or 3;

R is

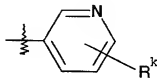


10

where

R^j is C_5 - C_{14} alkoxy or C_5 - C_{14} alkyl, and $p = 0, 1$ or 2 ;

R is



15

where

R^k is C_5 - C_{14} alkoxy; or

R is $-(CH_2)-NR^m$ -(C_{13} - C_{18} alkyl), where R^m is H, $-CH_3$ or

-C(O)CH₃; and

pharmaceutically acceptable salts and solvates thereof.

In another embodiment of the present invention, a pharmaceutical formulation is provided which includes the
5 pseudomycin compound represented by structure I above and a pharmaceutically acceptable carrier.

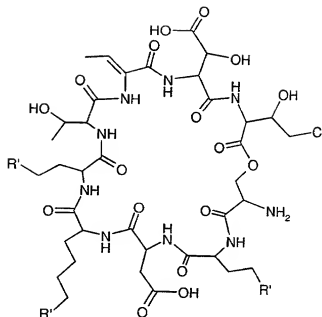
In yet another embodiment of the present invention, a method is provided for treating an antifungal infection in an animal in need thereof, which comprises administering to
10 the animal the pseudomycin compound I described above.

In yet another embodiment of the present invention, a process is provided for producing the free amine nucleus of a pseudomycin compound which may be acylated to form the compounds represented by structure I above. The process
15 includes the steps of treating a pseudomycin compound which contains an N-acyl alkyl side-chain having at least one gamma or delta hydroxyl group (e.g., pseudomycin A, A' or C) with trifluoroacetic acid or acetic acid.

20

Definitions

As used herein, the term "free amine pseudomycin nucleus" or "pseudomycin nucleus" refers to the structure I-A below:

I-A

wherein R' is -NH₂ or -NHp-Pg where Pg is an amino protecting group and p is 0 or 1.

- 5 The term "alkyl" refers to a hydrocarbon radical of the general formula C_nH_{2n+1} containing from 1 to 30 carbon atoms unless otherwise indicated. The alkane radical may be straight (e.g. methyl, ethyl, propyl, butyl, etc.), branched (e.g., isopropyl, isobutyl, tertiary butyl, neopentyl, etc.), cyclic (e.g., cyclopropyl, cyclobutyl, cyclopentyl, methylcyclopentyl, cyclohexyl, etc.), or multi-cyclic (e.g., bicyclo[2.2.1]heptane, spiro[2.2]pentane, etc.). The alkane radical may be substituted or unsubstituted. Similarly, the alkyl portion of an alkoxy group, alkanoyl, or alkanoate
- 10
- 15 have the same definition as above.

The term "alkenyl" refers to an acyclic hydrocarbon containing at least one carbon carbon double bond. The alkene radical may be straight, branched, cyclic, or multi-cyclic. The alkene radical may be substituted or
5 unsubstituted. The alkenyl portion of an alkenoxy, alkenoyl or alkenoate group has the same definition as above.

The term "alkynyl" refers to an acyclic hydrocarbon containing at least one carbon carbon triple bond. The alkyne radical may be straight, or branched. The alkyne
10 radical may be substituted or unsubstituted. The alkynyl portion of an alkynoxy, alkynoyl or alkynoate group has the same definition as above.

The term "aryl" refers to aromatic moieties having single (e.g., phenyl) or fused ring systems (e.g.,
15 naphthalene, anthracene, phenanthrene, etc.). The aryl groups may be substituted or unsubstituted.

The term "heteroaryl" refers to aromatic moieties containing at least one heteratom within the aromatic ring system (e.g., pyrrole, pyridine, indole, thiophene, furan,
20 benzofuran, imidazole, pyrimidine, purine, benzimidazole, quinoline, etc.). The aromatic moiety may consist of a single or fused ring system. The heteroaryl groups may be substituted or unsubstituted.

"NH_P-Pg" and "amino protecting group" refer to a
25 substituent of the amino group commonly employed to block or

protect the amino functionality while reacting other functional groups on the compound. When p is 0, the amino protecting group, when taken with the nitrogen to which it is attached, forms a cyclic imide, e.g., phthalimido and tetrachlorophthalimido. When p is 1, the protecting group, when taken with the nitrogen to which it is attached, can form a carbamate, e.g., methyl, ethyl, and 9-fluorenylmethylcarbamate; or an amide, e.g., N-formyl and N-acetylamide.

- 10 Within the field of organic chemistry and particularly within the field of organic biochemistry, it is widely understood that significant substitution of compounds is tolerated or even useful. In the present invention, for example, the term alkyl group allows for substituents which
- 15 is a classic alkyl, such as methyl, ethyl, propyl, hexyl, isooctyl, dodecyl, stearyl, etc. The term "group" specifically envisions and allows for substitutions on alkyls which are common in the art, such as hydroxy, halogen, alkoxy, carbonyl, keto, ester, carbamate, etc., as
- 20 well as including the unsubstituted alkyl moiety. However, it is generally understood by those skilled in the art that the substituents should be selected so as to not adversely affect the pharmacological characteristics of the compound or adversely interfere with the use of the medicament.
- 25 Suitable substituents for any of the groups defined above

include alkyl, alkenyl, alkynyl, aryl, halo, hydroxy, alkoxy, aryloxy, mercapto, alkylthio, arylthio, mono- and di-alkyl amino, quaternary ammonium salts, aminoalkoxy, hydroxyalkylamino, aminoalkylthio, carbamyl, carbonyl, 5 carboxy, glycolyl, glycyl, hydrazino, guanyl, and combinations thereof.

The term "solvate" refers to an aggregate that comprises one or more molecules of the solute, such as a compound of structure I, with one or more molecules of a pharmaceutical solvent, such as water, ethanol, and the like. 10

The term "pharmaceutically acceptable salt" refers to organic or inorganic salts of the compounds represented by structure I that are substantially non-toxic to the recipient at the doses administered. 15

The term "animal" refers to humans, companion animals (e.g., dogs, cats and horses), food-source animals (e.g., cows, pigs, sheep and poultry), zoo animals, marine animals, birds and other similar animal species.

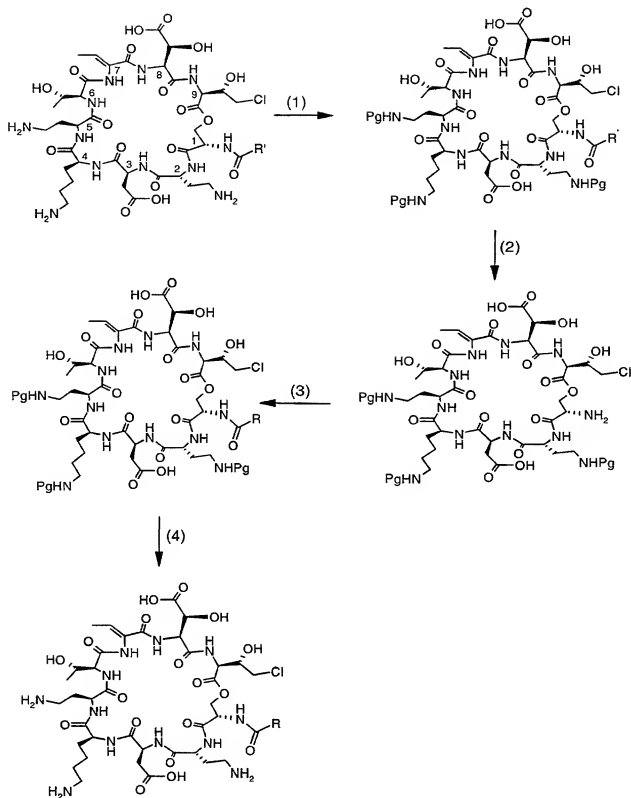
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DETAILED DESCRIPTION OF THE INVENTION

Applicants have discovered that deacylation of the N-acyl group of the L-serine unit of a pseudomycin compound followed by reacylation with a new N-acyl group provides 25 compounds having *in vitro* indications which suggest that the

new compounds may be active against *C. albican*, *C. neoformans*, and/or *Aspergillus fumigatus*.

Scheme I below illustrates the general procedures for synthesizing Compound I from any one of the naturally occurring pseudomycins. Although a naturally occurring pseudomycin compound is depicted in scheme I, those skilled in the art will understand that side-chain modification of semi-synthetic derivatives of the naturally occurring pseudomycin compounds may be accomplished in a similar manner. In general, four synthetic steps are used to produce Compound I: (1) selective amino protection; (2) chemical or enzymatic deacylation of the N-acyl side-chain; (3) reacylation with a different side-chain; and (4) deprotection of the amino groups.



Scheme I

The pendant amino groups at positions 2, 4 and 5 may be protected using any standard means known to those skilled in the art for amino protection. The exact genus and species of amino protecting group employed is not critical so long as the derivatized amino group is stable to the conditions of subsequent reaction(s) on other positions of the intermediate molecule and the protecting group can be selectively removed at the appropriate point without disrupting the remainder of the molecule including any other amino protecting group(s). Suitable amino-protecting groups include benzyloxycarbonyl, *p*-nitrobenzyloxycarbonyl, *p*-bromobenzyloxycarbonyl, *p*-methoxybenzyloxycarbonyl, *p*-methoxyphenylazobenzyloxycarbonyl, *p*-phenylazobenzyloxycarbonyl, *t*-butyloxycarbonyl, cyclopentyloxycarbonyl, and phthalimido. Preferred amino protecting groups are *t*-butoxycarbonyl (*t*-Boc), allyloxycarbonyl (Alloc), phthalimido, and benzyloxycarbonyl (CbZ or CBZ). Most preferred is allyloxycarbonyl and benzyloxycarbonyl. Further examples of suitable protecting groups are described in T.W. Greene, "Protective Groups in Organic Synthesis," John Wiley and Sons, New York, N.Y., (2nd ed., 1991), at chapter 7.

The deacylation of a N-acyl group having a gamma or delta hydroxylated side chain (e.g., 3,4-dihydroxytetra- deconoate) may be accomplished by treating the amino-

protected pseudomycin compound with a 5-20% aqueous acidic solution. Suitable acids include acetic acid and trifluoroacetic acid. A preferred acid is trifluoroacetic acid. If trifluoroacetic acid is used, the reaction may be accomplished at or near room temperature. However, when acetic acid is used the reaction is generally run at about 40°C. A water soluble organic solvent may be used to assist in solubilizing the pseudomycin compound. Suitable aqueous solvent systems include acetonitrile, water, and mixtures thereof. Acetonitrile was particularly useful when deacylating a protected pseudomycin compound. A preferred acidic solution for deacylating a protected pseudomycin compound is 8% aqueous trifluoroacetic acid in acetonitrile. Organic solvents accelerate the reaction; however, the addition of an organic solvent may lead to other by-products. Pseudomycin compounds lacking a delta hydroxy group on the side chain (e.g., Pseudomycin B and C') may be deacylated enzymatically. Suitable deacylase enzymes include Polymyxin Acylase (164-16081 Fatty Acylase (crude) or 161-16091 Fatty Acylase (pure) available from Wako Pure Chemical Industries, Ltd.), or ECB deacylase (see, e.g., U.S. Patent No. 5,573,936). The enzymatic deacylation may be accomplished using standard deacylation procedures well known to those skilled in the art. For example, general procedures for using Polymyxin acylase may be found in

Yasuda, N., et al., Agric. Biol. Chem., 53, 3245 (1989) and Kimura, Y., et al., Agric. Biol. Chem., 53, 497 (1989).

The deacylated product (also known as the pseudomycin nucleus or "PSN") is reacylated using the corresponding acid of the desired acyl group in the presence of a carbonyl activating agent. "Carbonyl activating group" refers to a substituent of a carbonyl that promotes nucleophilic addition reactions at that carbonyl. Suitable activating substituents are those which have a net electron withdrawing effect on the carbonyl. Such groups include, but are not limited to, alkoxy, aryloxy, nitrogen containing aromatic heterocycles, or amino groups (e.g., oxybenzotriazole, imidazolyl, nitrophenoxy, pentachlorophenoxy, N-oxy succinimide, N,N'-dicyclohexylisoure-O-yl, and N-hydroxy-N-methoxyamino); acetates; formates; sulfonates (e.g., methanesulfonate, ethanesulfonate, benzenesulfonate, and p-tolylsulfonate); and halides (e.g., chloride, bromide, and iodide).

Alternatively, a solid phase synthesis may be used where a hydroxybenzotriazole-resin (HOBt-resin) serves as the coupling agent for the acylation reaction.

A variety of acids may be used in the acylation process. Suitable acids include aliphatic acids containing one or more pendant aryl, alkyl, amino (including primary, secondary and tertiary amines), hydroxy, alkoxy, and amido

groups; aliphatic acids containing nitrogen or oxygen within the aliphatic chain; aromatic acids substituted with alkyl, hydroxy, alkoxy and/or alkyl amino groups; and heteroaromatic acids substituted with alkyl, hydroxy, alkoxy and/or alkyl amino groups. The acylated product may be useful as an active antifungal agent or as an intermediate for the production of an active compound. Even though some compounds were not as useful as others, the activity profiles provide valuable insight into the design trends needed to achieve optimum activity.

Once the amino group is acylated, then the amino protecting groups (at positions 2, 4 and 5) may be removed by hydrogenation in the presence of a hydrogenation catalyst (e.g., 10% Pd/C). When the amino protecting group is allyloxycarbonyl, then the protecting group may be removed using tributyltinhydride and triphenylphosphine palladium dichloride. This particular protection/deprotection scheme has the advantage of reducing the potential for hydrogenating the vinyl group of the Z-Dhb unit of the pseudomycin structure.

As discussed earlier, pseudomycins are natural products isolated from the bacterium *Pseudomonas syringae* that have been characterized as lipodepsinonapeptides containing a cyclic peptide portion closed by a lactone bond and including the unusual amino acids 4-chlorothreonine (ClThr),

3-hydroxyaspartic acid (HOAsp), 2,3-dehydro-2-aminobutyric acid (Dhb), and 2,4-diaminobutyric acid (Dab). Methods for growth of various strains of *P. syringae* to produce the different pseudomycin analogs (A, A', B, B', C, and C') are described below and described in more detail in PCT Patent Application Serial No. PCT/US00/08728 filed by Hilton, et al. on April 14, 2000 entitled "Pseudomycin Production by *Pseudomonas Syringae*," incorporated herein by reference, PCT Patent Application Serial No. PCT/US00/08727 filed by Kulanthaivel, et al. on April 14, 2000 entitled "Pseudomycin Natural Products," incorporated herein by reference, and U.S. Patent Nos. 5,576,298 and 5,837,685, each of which are incorporated herein by reference.

Isolated strains of *P. syringae* that produce one or more pseudomycins are known in the art. Wild type strain MSU 174 and a mutant of this strain generated by transposon mutagenesis, MSU 16H (ATCC 67028) are described in U.S. Patent Nos. 5,576,298 and 5,837,685; Harrison, et al., "Pseudomycins, a family of novel peptides from *Pseudomonas syringae* possessing broad-spectrum antifungal activity," J. Gen. Microbiology, **137**, 2857-2865 (1991); and Lamb et al., "Transposon mutagenesis and tagging of fluorescent pseudomonas: Antimycotic production is necessary for control

of Dutch elm disease," Proc. Natl. Acad. Sci. USA, **84**, 6447-6451 (1987).

A strain of *P. syringae* that is suitable for production of one or more pseudomycins can be isolated from

5 environmental sources including plants (e.g., barley plants, citrus plants, and lilac plants) as well as, sources such as soil, water, air, and dust. A preferred stain is isolated from plants. Strains of *P. syringae* that are isolated from environmental sources can be referred to as wild type. As
10 used herein, "wild type" refers to a dominant genotype which naturally occurs in the normal population of *P. syringae* (e.g., strains or isolates of *P. syringae* that are found in nature and not produced by laboratory manipulation). Like most organisms, the characteristics of the pseudomycin-
15 producing cultures employed (*P. syringae* strains such as MSU 174, MSU 16H, MSU 206, 25-B1, 7H9-1) are subject to variation. Hence, progeny of these strains (e.g., recombinants, mutants and variants) may be obtained by methods known in the art.

20 *P. syringae* MSU 16H is publicly available from the American Type Culture Collection, Parklawn Drive, Rockville, MD, USA as Accession No. ATCC 67028. *P. syringae* strains 25-B1, 7H9-1, and 67 H1 were deposited with the American Type Culture Collection on March 23, 2000 and were assigned
25 the following Accession Nos.:

25-B1	Accession No. PTA-1622
7H9-1	Accession No. PTA-1623
67 H1	Accession No. PTA-1621

Mutant strains of *P. syringae* are also suitable for

5 production of one or more pseudomycins. As used herein, "mutant" refers to a sudden heritable change in the phenotype of a strain, which can be spontaneous or induced by known mutagenic agents, such as radiation (e.g., ultraviolet radiation or x-rays), chemical mutagens (e.g.,

10 ethyl methanesulfonate (EMS), diepoxyoctane, N-methyl-N-nitro-N'-nitrosoguanine (NTG), and nitrous acid), site-specific mutagenesis, and transposon mediated mutagenesis. Pseudomycin-producing mutants of *P. syringae* can be produced by treating the bacteria with an amount of a mutagenic agent

15 effective to produce mutants that overproduce one or more pseudomycins, that produce one pseudomycin (e.g., pseudomycin B) in excess over other pseudomycins, or that produce one or more pseudomycins under advantageous growth conditions. While the type and amount of mutagenic agent to

20 be used can vary, a preferred method is to serially dilute NTG to levels ranging from 1 to 100 µg/ml. Preferred mutants are those that overproduce pseudomycin B and grow in minimal defined media.

Environmental isolates, mutant strains, and other

25 desirable strains of *P. syringae* can be subjected to

selection for desirable traits of growth habit, growth medium nutrient source, carbon source, growth conditions, amino acid requirements, and the like. Preferably, a pseudomycin producing strain of *P. syringae* is selected for growth on minimal defined medium such as N21 medium and/or for production of one or more pseudomycins at levels greater than about 10 µg/ml. Preferred strains exhibit the characteristic of producing one or more pseudomycins when grown on a medium including three or fewer amino acids and optionally, either a lipid, a potato product or combination thereof.

Recombinant strains can be developed by transforming the *P. syringae* strains, using procedures known in the art. Through the use of recombinant DNA technology, the *P. syringae* strains can be transformed to express a variety of gene products in addition to the antibiotics these strains produce. For example, one can modify the strains to introduce multiple copies of the endogenous pseudomycin-biosynthesis genes to achieve greater pseudomycin yield.

To produce one or more pseudomycins from a wild type or mutant strain of *P. syringae*, the organism is cultured with agitation in an aqueous nutrient medium including an effective amount of three or fewer amino acids, preferably glutamic acid, glycine, histidine, or a combination thereof.

Alternatively, glycine is combined with one or more of a potato product and a lipid. Culturing is conducted under conditions effective for growth of *P. syringae* and production of the desired pseudomycin or pseudomycins.

- 5 Effective conditions include temperatures from about 22°C to about 27°C, and a duration of about 36 hours to about 96 hours. Controlling the concentration of oxygen in the medium during culturing of *P. syringae* is advantageous for production of a pseudomycin. Preferably, oxygen levels are
- 10 maintained at about 5 to 50% saturation, more preferably about 30% saturation. Sparging with air, pure oxygen, or gas mixtures including oxygen can regulate the concentration of oxygen in the medium.

- Controlling the pH of the medium during culturing of *P.*
- 15 *syringae* is also advantageous. Pseudomycins are labile at basic pH, and significant degradation can occur if the pH of the culture medium is above about 6 for more than about 12 hours. Preferably, the pH of the culture medium is maintained between 6 and 4. *P. syringae* can produce one or
- 20 more pseudomycins when grown in batch culture. However, fed-bath or semi-continuous feed of glucose and optionally, an acid or base (e.g., ammonium hydroxide) to control pH, enhances production. Pseudomycin production can be further enhanced by using continuous culture methods in which
- 25 glucose and ammonium hydroxide are fed automatically.

Choice of *P. syringae* strain can affect the amount and distribution of pseudomycin or pseudomycins produced. For example, strains MSU 16H and 67 H1 each produce predominantly pseudomycin A, but also produce pseudomycin B and C, typically in ratios of 4:2:1. Strain 67 H1 typically produces levels of pseudomycins about three to five fold larger than are produced by strain MSU 16H. Compared to strains MSU 16H and 67 H1, strain 25-B1 produces more pseudomycin B and less pseudomycin C. Strain 7H9-1 are distinctive in producing predominantly pseudomycin B and larger amount of pseudomycin B than other strains. For example, this strain can produce pseudomycin B in at least a ten fold excess over either pseudomycin A or C.

Each pseudomycin, pseudomycin intermediate and mixtures can be detected, determined, isolated, and/or purified by any variety of methods known to those skilled in the art. For example, the level of pseudomycin activity in a broth or in an isolate or purified composition can be determined by antifungal action against a fungus such as *Candida* and can be isolated and purified by high performance liquid chromatography.

The pseudomycin compound may be isolated and used *per se* or in the form of its pharmaceutically acceptable salt or solvate. The term "pharmaceutically acceptable salt" refers to non-toxic acid addition salts derived from inorganic and

- organic acids. Suitable salt derivatives include halides, thiocyanates, sulfates, bisulfates, sulfites, bisulfites, arylsulfonates, alkylsulfates, phosphonates, monohydrogen-phosphates, dihydrogenphosphates, metaphosphates,
- 5 pyrophosphonates, alkanoates, cycloalkylalkanoates, arylalkonates, adipates, alginates, aspartates, benzoates, fumarates, glucoheptanoates, glycerophosphates, lactates, maleates, nicotines, oxalates, palmitates, pectinates, picrates, pivalates, succinates, tartarates, citrates,
- 10 camphorates, camphorsulfonates, digluconates, trifluoroacetates, and the like.

- The term "solvate" refers to an aggregate that comprises one or more molecules of the solute (i.e., pseudomycin prodrug compound) with one or more molecules of
- 15 a pharmaceutical solvent, such as water, ethanol, and the like. When the solvent is water, then the aggregate is referred to as a hydrate. Solvates are generally formed by dissolving the compound in the appropriate solvent with heat and slowing cooling to generate an amorphous or crystalline
- 20 solvate form.

- The active ingredient (i.e., pseudomycin derivative) is typically formulated into pharmaceutical dosage forms to provide an easily controllable dosage of the drug and to give the physician, patient, or veterinarian an elegant and
- 25 easily handleable product. Formulations may comprise from

0.1% to 99.9% by weight of active ingredient, more generally from about 10% to about 30% by weight.

As used herein, the term "unit dose" or "unit dosage" refers to physically discrete units that contain a

5 predetermined quantity of active ingredient calculated to produce a desired therapeutic effect. When a unit dose is administered orally or parenterally, it is typically provided in the form of a tablet, capsule, pill, powder packet, topical composition, suppository, wafer, measured
10 units in ampoules or in multidose containers, etc.

Alternatively, a unit dose may be administered in the form of a dry or liquid aerosol which may be inhaled or sprayed.

The dosage to be administered may vary depending upon the physical characteristics of the animal, the severity of
15 the animal's symptoms, and the means used to administer the drug. The specific dose for a given animal is usually set by the judgment of the attending physician or veterinarian.

Suitable carriers, diluents and excipients are well known to those skilled in the art and include materials such
20 as carbohydrates, waxes, water soluble and/or swellable polymers, hydrophilic or hydrophobic materials, gelatin, oils, solvents, water, and the like. The particular carrier, diluent or excipient used will depend upon the means and purpose for which the active ingredient is being
25 applied. The formulations may also include wetting agents,

lubricating agents, surfactants, buffers, tonicity agents, bulking agents, stabilizers, emulsifiers, suspending agents, preservatives, sweeteners, perfuming agents, flavoring agents and combinations thereof.

5 A pharmaceutical composition may be administered using a variety of methods. Suitable methods include topical (e.g., ointments or sprays), oral, injection and inhalation. The particular treatment method used will depend upon the type of infection being addressed.

10 In parenteral iv applications, the formulations are typically diluted or reconstituted (if freeze-dried) and further diluted if necessary, prior to administration. An example of reconstitution instructions for the freeze-dried product are to add ten ml of water for injection (WFI) to
15 the vial and gently agitate to dissolve. Typical reconstitution times are less than one minute. The resulting solution is then further diluted in an infusion solution such as dextrose 5% in water (D5W), prior to administration.

20 Pseudomycin compounds have been shown to exhibit antifungal activity such as growth inhibition of various infectious fungi including *Candida* spp. (i.e., *C. albicans*, *C. parapsilosis*, *C. krusei*, *C. glabrata*, *C. tropicalis*, or *C. lusitania*); *Torulopus* spp. (i.e., *T. glabrata*);
25 *Aspergillus* spp. (i.e., *A. fumigatus*); *Histoplasma* spp.

(i.e., *H. capsulatum*); *Cryptococcus* spp. (i.e., *C. neoformans*); *Blastomyces* spp. (i.e., *B. dermatitidis*); *Fusarium* spp.; *Trichophyton* spp., *Pseudallescheria boydii*, *Coccidioides immitis*, *Sporothrix schenckii*, etc.

- 5 Consequently, the compounds and formulations of the present invention may be useful in the preparation of medicaments for use in combating either systemic fungal infections or fungal skin infections. Accordingly, a method is provided for inhibiting fungal activity comprising
- 10 contacting Compound I of the present invention with a fungus. A preferred method includes inhibiting *Candida albicans*, *Cryptococcus neoformans*, or *Aspergillus fumigatus* activity. The term "contacting" includes a union or junction, or apparent touching or mutual tangency of a
- 15 compound of the invention with a fungus. The term does not imply any further limitations to the process, such as by mechanism of inhibition. The methods are defined to encompass the inhibition of fungal activity by the action of the compounds and their inherent antifungal properties.
- 20 A method for treating a fungal infection which comprises administering an effective amount of a pharmaceutical formulation of the present invention to a host in need of such treatment is also provided. A preferred method includes treating a *Candida albicans*,
- 25 *Cryptococcus neoformans*, or *Aspergillus fumigatus* infection.

The term "effective amount" refers to an amount of active compound which is capable of inhibiting fungal activity.

The dose administered will vary depending on such factors as the nature and severity of the infection, the age and

- 5 general health of the host, the tolerance of the host to the antifungal agent and the species of the host. The particular dose regimen likewise may vary according to these factors. The medicament may be given in a single daily dose or in multiple doses during the day. The regimen may last
- 10 from about 2-3 days to about 2-3 weeks or longer. A typical daily dose (administered in single or divided doses) contains a dosage level between about 0.01 mg/kg to 100 mg/kg of body weight of an active compound. Preferred daily doses are generally between about 0.1 mg/kg to 60 mg/kg and
- 15 more preferably between about 2.5 mg/kg to 40 mg/kg. The host may be any animal including humans, companion animals (e.g., dogs, cats and horses), food-source animals (e.g., cows, pigs, sheep and poultry), zoo animals, marine animals, birds and the like.

20

EXAMPLES

Unless indicated otherwise, all chemicals can be acquired from Aldrich Chemical (Milwaukee, WI).

Biological Samples

- P. *syringae* MSU 16H is publicly available from the
- 25 American Type Culture Collection, Parklawn Drive, Rockville,

MD, USA as Accession No. ATCC 67028. *P. syringae* strains 25-B1, 7H9-1, and 67 H1 were deposited with the American Type Culture Collection on March 23, 2000 and were assigned the following Accession Nos.:

5	25-B1	Accession No. PTA-1622
	7H9-1	Accession No. PTA-1623
	67 H1	Accession No. PTA-1621

Chemical Abbreviations

The following abbreviations are used through out the examples to represent the respective listed materials:

10	ACN - acetonitrile
	TFA - trifluoroacetic acid
	DMF - dimethylformamide
15	DEAD - Diethylazodicarboxylate
	EDCI - 1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide hydrochloride
	BOC = t-butoxycarbonyl, $(\text{CH}_3)_3\text{C}-\text{O}-\text{C}(\text{O})-$
	CBZ = benzyloxycarbonyl, $\text{C}_6\text{H}_5\text{CH}_2-\text{O}-\text{C}(\text{O})-$
20	FMOC = fluorenylmethyloxycarbonyl

HPLC Conditions

Unless indicated otherwise, analytical reverse-phase HPLC work was done using the Waters 600E systems equipped with Waters μ Bondapak (C18, 3.9 X 300 mm) column. The eluent used was 65:35 acetonitrile/0.1% aqueous TFA solvent

system to 100% acetonitrile over 20 minutes with a flow rate of 1.5 ml/minute and using UV detection at 230 nm.

- Preparative HPLC work was performed with a Waters Prep 2000 system using Dynamax 60 angstrom C18 column and
- 5 identical solvent systems as used in the analytical HPLC system but with a flow rate of 40 ml/min.

Biological Analysis

Detection and Quantification of Antifungal Activity:

- Antifungal activity was determined *in vitro* by
- 10 obtaining the minimum inhibitory concentration (MIC) of the compound using a standard agar dilution test or a disc-diffusion test. A typical fungus employed in testing antifungal activity is *Candida albicans*. Antifungal activity is considered significant when the test sample (50
- 15 μ l) causes 10-12 mm diameter zones of inhibition on *C. albicans* x657 seeded agar plates.

Tail Vein Toxicity:

- Mice were treated intravenously (IV) through the
- 20 lateral tail vein with 0.1 ml of testing compound (20 mg/kg) at 0, 24, 48 and 72 hours. Two mice were included in each group. Compounds were formulated in 5.0% dextrose and sterile water for injection. The mice were monitored for 7 days following the first treatment and observed closely for

signs of irritation including erythema, swelling, discoloration, necrosis, tail loss and any other signs of adverse effects indicating toxicity.

- The mice used in the study were outbred, male ICR mice
- 5 having an average weight between 18-20 g (available from Harlan Sprague Dawley, Indianapolis, IN).

General Procedures

General procedures used to protect the pendant amino groups at positions 2, 4 and 5 of Pseudomycin A, A', B, B', C or

10 C'.

- Dissolve/suspend pseudomycin compound ($R^1=H$) in DMF (20 mg/ml, Aldrich Sure Seal). While stirring at room temperature add *N*-(Benzyloxycarbonyloxy)succinimide (6 eq). Allow to stir at room temperature for 32 hours. Monitor
- 15 reaction by HPLC (4.6x50 mm, 3.5 μ m, 300-SB, C8, Zorbax column). Concentrate reaction to 10 ml on high vacuum rotovap at room temperature. Put material in freezer until ready to prep by chromatography. Reverse phase preparative HPLC yields an amorphous, white solid after lyophilization.

20

General procedures used for chemically deacylating the *N*-acyl group of the *L*-serine unit.

Dissolve/suspend protected Pseudomycin A in water/acetonitrile (2:1 H₂O:ACN, about 3.5 mg/ml) and add

TFA (8% by volume) slowly at room temperature. Allow the reaction to stir at room temperature until starting material is consumed. Remove acetonitrile under vacuum at room temperature and lyophilize the material. Dissolve resulting solid in a small amount of DMF (add water and then an equal amount of ACN if necessary). After preparative HPLC and lyophilization, a white, amorphous solid (TFA salt, presumably) is generally observed.

- 10 Solid phase acylation of the Pseudomycin nucleus using HOBt-resin. The following example uses myristoyl acid; however, the same general procedure may be used with other organic acids.

In a 100 ml double-ended glass fritted reaction tube, myristoyl acid (1.03 g, 3.62 mmol) was dissolved in 50 ml 1:1 DMF/THF. To this solution was added resin HOBt (1.94g, 2.9 mmol), and EDCI (0.556 g, 2.9 mmol) and was shaken overnight. The solvent was drained and the resin was washed with 2xDMF, 2xTHF, and 2x with 1:1 DMF/THF. CBZ-protected Pseudomycin nucleus (1.0 grams, 0.723 mmol) was dissolved in 50 ml DMF/THF (20 mg/ml), and added to the resin bound activated ester and mixed overnight on a rotator or shaker. The product was drained away from the resin and the remaining resin was washed with 2xDMF, 2xTHF, and 2x1:1 DMF/THF. The combined filtrates were isolated by reverse

phase HPLC and lyophilized to yield (129 mg, 10%) Myristoyl acylated CBZ-protected Pseudomycin product.

Acylation of the Pseudomycin nucleus using an activated

- 5 ester (HOBt-mesylate). The following example uses glycine myristoyl acid; however, the general procedures may be used with other organic acids.

In a 500 ml round bottom flask, glycine myristoyl acid (0.309 g, 1.1 mmol) was dissolved in 100 ml of DMF. To this
10 solution was added HOBt-mesylate (0.229 g, 1.1 mmol) and triethylamine (0.081g, 0.8mmol) The solution was stirred rapidly overnight under 1 atm N₂. DMF and TEA were dried off using the high vacuum. The residual oil was azeotroped 3x with toluene till a white solid formed. To the solid was
15 added 100 ml of DMF and 1g of CBZ-protected Pseudomycin nucleus. The solution was stirred overnight, and dried on the high vacuum. The product was isolated by reverse-phase HPLC and lyophilized to yield (233 mg, 20%) Myristoyl acylated CBZ-protected Pseudomycin product.

20

General procedures used to deprotect the pendant amino groups at position 2, 4 and 5 by hydrogenation.

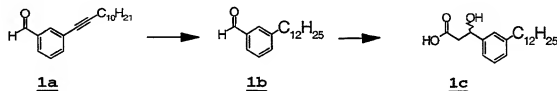
Dissolve CBZ-protected acylated-derivative in a cold 13% acetic/methanol solution (5 mg/ml) and add an equivalent
25 amount of 10% Pd/C. Charge the reaction with hydrogen by

degassing reaction and replacing volume with H₂, 4-7 times. Allow reaction to proceed at room temperature. Monitor the reaction by HPLC and mass spectrometry every 15 minutes until the starting material is consumed. When the reaction is complete, remove balloon and filter reaction with 0.45 μm filter disk (Acrodisk GHP, GF by Gelman). Concentrate to about 1/10th volume and prep by HPLC. Lyophilize fractions containing product.

10

Preparations

Preparation of Side-Chain Precursor (1c):



15

A 250 ml round bottom flask containing 100 ml of degassed ACN was charged with *m*-bromobenzaldehyde (5.000 g, 27.02 mmol), triethylamine (5.490 g, 54.25 mmol) and 1-dodecyne (5.000g, 30.06 mmol). To this mixture was added PdCl₂ (243.1 mg, 1.370 mmol), triphenylphosphine (718.8 mg, 2.740 mmol) and CuI (173.8 mg, 0.9120 mmol). The reaction was then heated to reflux and allowed to react overnight. The reaction was then cooled to room temperature and the solvent was removed *in vacuo*. The resulting residue was taken up in methylene chloride and washed 2 X 1N HCl and 1 X

brine. The organic layer was dried over MgSO_4 . The drying agent was then filtered off and the solvent was removed in vacuo. Purification on a silica gel column eluting with 3% EtOAc/hexanes yielded 3.73 grams of a the titled compound as
5 a brown oil. The spectral data was consistent with the structure for *m*-(1-Dodecynyl)benzaldehyde (1a).

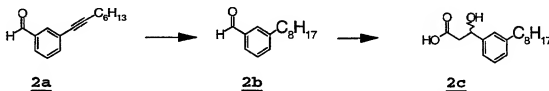
To 100 mL of EtOAc was added the above compound (1.00 g, 3.70 mmol) and 0.1 g of 5% $\text{Pd/Al}_2\text{O}_3$. The reaction mixture was subjected to 50psi of H_2 at room temperature for 1 hour.
10 The reaction mixture was filtered over celite to remove the catalyst and the celite was rinsed with copious amounts of EtOAc. Removal of the EtOAc via a rotary evaporator yielded 882.1 mg of the product. This was used in the next step without further purification. The spectral data was
15 consistent with *m*-Dodecylbenzaldehyde (1b).

An oven dried 50 ml round bottom flask was charged with 6.0 ml anhydrous THF under a nitrogen atmosphere at -78°C and lithium diisopropyl amine (1.2 mL of a 2M solution in heptane/THF/ethylbenzene, 2.41 mmol) was then added. To
20 this was added *t*-butyl acetate (0.331 mL, 2.45 mmol) and the resulting solution was raised to approx. -40°C and maintained at this temperature for 1 hour. The above compound (501.9mg, 1.83 mmol), dissolved in 4 mL anhydrous THF and precooled to -40°C , was then added dropwise to the
25 anion. The reaction mixture was allowed to stir for 1 hour

before quenching with 2 ml of saturated aqueous ammonium chloride and 10 ml water. The reaction mixture was partitioned between ether and water. The organic layer was washed 1 X brine and dried over sodium sulfate. The drying agent was then filtered off and the solvent was removed *in vacuo*. Purification on a silica gel column eluting with 5% EtOAc/hexanes yielded 238.1 mg of a yellow oil. The spectral data was consistent with *t*-Butyl 3-hydroxy-3-(*m*-dodecylbenzyl) propionate.

- 10 A solution of precooled (0°C) of 4 ml TFA was added to a 50 ml round bottom flask containing crude *t*-Butyl 3-hydroxy-3-(*m*-dodecylbenzyl) propionate. The reaction was allowed to stir at this temperature for 25 min at which time TLC (10% EtOAc/hexanes) indicated the consumption of the
- 15 starting ester. The TFA was removed *in vacuo* yielding an oil (1c).

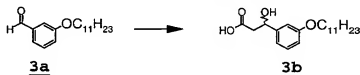
Preparation of Side-Chain Precursor (2c):



Compound 2a is synthesized using the same procedures as described above for Compound 1a using 1-octyne instead of 1-

dodecyne. Compounds 2b and 2c are synthesized using the same procedures described above for 1b and 1c, respectively.

Preparation of Side-Chain Precursor (3b):



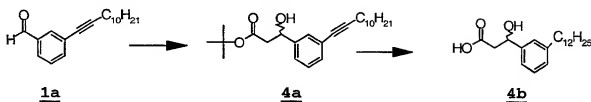
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A 500 mL round bottom flask containing 100 mL of acetone was charged with *m*-hydroxybenzaldehyde (5.00g, 40.94 mmol), 1-bromoundecane (9.65g, 41.02 mmol) and K₂CO₃ (8.48g, 61.36 mmol). The reaction mixture was heated to reflux and allowed to react for 10 h. The reaction was then cooled and the acetone was removed *in vacuo*. The resulting residue was partitioned between ether/water. The organic layer was washed 2 X saturated aqueous NaHCO₃ and 1 X Brine. The organic layer was dried over MgSO₄. The drying agent was then filtered off and the solvent was removed *in vacuo*. Purification on a silica gel column eluting with 3% EtOAc/hexanes yielded 1.6876 g of a yellow oil. The spectral data was consistent with Compound **3a**. This aldehyde was then carried through using the same procedures described in the preparation of **1c** to produce Compound **3b**.

10

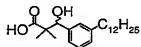
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Preparation of Side-Chain Precursor (4c):

Compound 4a is synthesized using the same procedures as described above for Compound 1c with the exception that Compound 1a is not hydrogenated prior to condensation with *t*-butyl acetate.

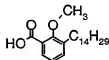
A 50 ml round bottom flask was charged with *t*-Butyl 3-hydroxy-3-(*m*-dodecynylbenzyl) propionate 4a (346.8 mg, 0.897) and dissolved in 10 ml MeOH / 1 mL glacial AcOH. Standard hydrogenolysis with 10% Pd/C (304.5 mg) for 24 h. Removal of the catalyst via filtration and removal of the solvent *in vacuo* led to 302.4 mg of *t*-Butyl 3-(*m*-dodecylbenzyl) propionate. The *t*-butyl ester was then removed by treatment with TFA to produce Compound 4b.

Preparation of Side-Chain Precursor (5a):

To a 50 ml round bottom flask containing 5 mL THF at -78°C was added *sec*-BuLi (1.56 mmol, 1.2 mL of a 1.3 M sol'n in cyclohexane). To this mixture was added *t*-butyl

- bromoisobutyrate (in 2 ml THF at -78°C) dropwise. This reaction mixture was allowed to stir for 30 min at which time Compound **1b** (318.9 mg, 1.16 mmol in 2 ml THF at -78°C) was then added to the reaction mixture dropwise. The
- 5 resulting reaction mixture was allowed to stir at -78°C for 30 min and then raised to 0°C over a period of 30 min and allowed to stir at that temperature for 1.75 h. The reaction was then quenched with 2 ml of saturated aqueous ammonium chloride and allowed to warm to room temperature.
- 10 The reaction mixture was the partitioned between ether/water and the organics were washed 1x brine and dried over MgSO_4 . The drying agent was then filtered off and the solvent was removed in vacuo to yield 370.5 mg of crude material as a racemic mixture. The t-butyl ester was then removed by
- 15 treatment with TFA to produce Compound **5a**.

Preparation of Side-Chain Precursor (6a):

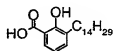


6a

- 20 To a 250 mL round bottom flask containing 100 mL MeOH and 1 mL of conc. H_2SO_4 was added *m*-bromoanisic acid (5.00 g, 21.6 mmol). The resulting mixture was heated to reflux and allowed to stir for 24 h. The reaction was cooled and the

solvent was removed in vacuo. The resulting solid was taken up in ether and the organics were washed 2x water, 1x saturated aqueous NaHCO₃ and 1x brine and dried over MgSO₄. The drying agent was then filtered off and the solvent was removed in vacuo to yield 4.72g of crude material which was used without further purification. This was then coupled 1-tetradecyne and underwent hydrogenolysis to the alkene analogously to previous examples. The methyl ester was converted to the carboxylic acid by suspending the methyl ester (538.4 mg, 1.48 mmol) in 20 ml of a 30% HBr in AcOH solution and heating to reflux. After 24 h at reflux the solution was poured into 150 ml of water and extracted out with 2x 200 ml CH₂Cl₂. The organics were washed with copious amounts of water and dried over MgSO₄. The drying agent was then filtered off and the solvent was removed in vacuo to yield 398.7g of crude material 6a which was used without further purification.

Preparation of Side-Chain Precursor (7a):

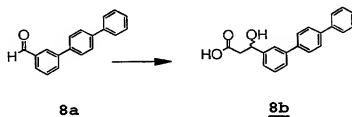


7a

Compound 6a (385.2 mg) was added to pyridinium hydrochloride solid. The 2 solids were melted by heating to

~ 220 °C and allowing the mixture to react for 3 h. The reaction was then cooled and partitioned between CH₂Cl₂/1N HCl. The organics were then washed 5x 1N HCl and dried over MgSO₄. The drying agent was then filtered off and the solvent was removed in vacuo to yield 158.8 mg of crude material (7a) which was used without further purification.

Preparation of Side-Chain Precursor (8b):



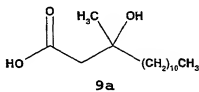
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A 250 ml round bottom flask was charged with biphenyl boronic acid (2.00 g, 10.1 mmol) and Pd(PPh)₄ (980.0 mg, 0.848 mmol) in 60 mL toluene/30 mL 2 M aqueous Na₂CO₃. To this slurry was added *m*-bromobenzaldehyde (in 10 mL MeOH). The reaction was heated to reflux and allowed to react for 20 h. The reaction was cooled and the organic layer was washed 2x water, 1x brine and dried over MgSO₄. The drying agent was then filtered off and the solvent was removed in vacuo. The resulting solids were rinsed with cold hexanes to remove any residual *m*-bromobenzaldehyde. The solid was then slurried in hot hexanes and filtered hot to remove any solids. The filtrate was then removed in vacuo to yield the desired aldehyde **8a**. Compound **8a** was converted to **8b** using

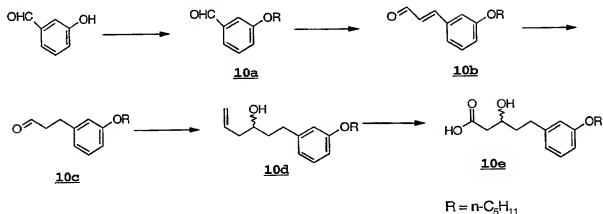
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the same procedures as described in the preparation of 1c to yield a mixture of inseparable diastereomers.

Preparation of Side-Chain Precursor (9a):



A solution of t-butyl acetate (2.02 ml, 0.015 mol) in anhydrous THF (25 ml) was cooled to -78 °C and n-butyl
10 lithium (1.6 M in hexane) (9.35 ml, 0.015 mol) was added dropwise. After 45 min a THF solution of 2-tridecanone (2.0 g, 0.01 mol) was added dropwise. The stirring was continued at low temperature for 1 hr and then the reaction was allowed to warm to room temperature over 15 min. Excess 1N
15 HCl was added to quench the reaction and the aqueous solution was extracted with ether (2x). The ether extracts were dried over MgSO₄ and reduced in vacuo to give a crude oil. Purification by column chromatography over silica (5% ethyl acetate/hexane) gave 1.01 g (32% yield) of the t-butyl
20 ester. NMR was consistent with the desired product. The t-butyl ester was then treated with trifluoroacetic acid to cleave the t-butyl ester to produce Compound 9a with a quantitative yield.

Preparation of Side-Chain Precursor (10e):

To a THF solution (16 mL) of m-hydroxybenzaldehyde (1.00 g, 8.20 mmol) was added at rt DEAD (1.29 mL, 8.20 mmol), PPh₃ (2.15 g, 8.20 mmol) and n-pentanol (723 mg, 8.20 mmol). The reaction was stirred overnight at rt. After silica gel purification, 1.02 g (65%) of the desired product **10a** was obtained.

To a THF solution of **10a** (6.70 g, 34.90 mmol) was added at 0°C Ph₃P=CHO (10.6 g, 34.90 mmol). After stirring at rt overnight, the reaction mixture was filtered and conc. in vacuo to give a residue, which was purified by silica gel chromatography to give 3.57 g (47%) of the desired unsaturated aldehyde **10b**.

An EtOAc solution (30 mL) of **10b** (3.37 g, 15.5 mmol) was subjected to hydrogenation (1.5 atm) using 10% Pd/C (1.64 g, 1.55 mmol). The reaction was stirred overnight. At this point, the reaction mixture was filtered through a pad of Celite. The filtrates and rinses were conc. in vacuo. The

residue thus obtained was purified by silica gel chromatography (10% EtOAc/Hexanes) to afford 2.37 g (70%) of 10c.

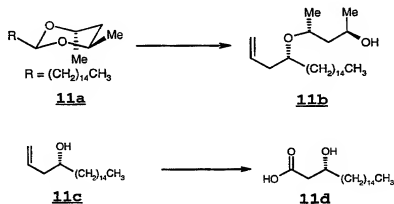
To a dichloromethane solution (23 mL) of aldehyde 10c
5 (1.26 g, 5.71 mmol) was added at 0°C allyltrimethylsilane (0.91 mL, 5.71 mmol), followed by TiCl₄ (0.63 mL, 5.71 mmol). After stirring for 1 hr at 0°C, the reaction was quenched with saturated NaHCO₃ solution. The mixture was diluted with dichloromethane (75 mL). The organic layer was
10 washed sequentially with saturated NaHCO₃, water and brine. The organic layer thus obtained was dried, conc. and purified via silica gel chromatography (10% EtOAc/Hexanes) to afford 0.91 g (61%) of the desired allylic alcohol 10d.

Allylic alcohol 10d (0.91 g, 3.47 mmol) was dissolved
15 in aqueous acetone (7 mL each). To this solution was added NMO (704 mg, 5.21 mmol) and a THF solution of OsO₄ (44 mg, 0.17 mmol). After stirring at rt for 2 hr, the reaction was quenched with NaHSO₃ (750 mg) to quench the excess oxidant. The reaction mixture was diluted with brine (10 mL) and
20 extracted with EtOAc (3 x 50 mL). The combined organic layers were dried and conc. in vacuo to provide 850 mg (82%) of the desired triol intermediate. The triol (850 mg, 2.87 mmol) thus obtained was dissolved in MeOH (30 mL) and water (6 mL). This solution was treated with NaIO₄ (1.38 g, 6.46

mmol) at rt. After 1 hr, the reaction mixture was filtered through a pad of Celite. The filtrates were carefully conc. in vacuo to yield the corresponding beta-hydroxy aldehyde. This material was dissolved in a mixture of t-BuOH (14 mL) and cyclohexene (2 mL). To the above solution was added an aqueous solution (15 mL H₂O) containing KH₂PO₄ (2.33 g, 17.7 mmol) and NaClO₂ (2.08 g, 23.0 mmol). The reaction was stirred at rt for 5 hr and then acidified to pH = 3 with 1N HCl. The reaction mixture was extracted with EtOAc (3 x 50 mL). The combined extracts were washed with water and brine. The organic layer was dried and conc. in vacuo to give the crude acid **10e** (1.5 g, ~3.4 mmol).

Compounds where R is n-C₆H₁₃, n-C₇H₁₅, n-C₈H₁₇, n-C₉H₁₉, n-C₁₀H₂₁, and n-C₁₄H₂₉ were also made using the same procedures as described above.

Preparation of Side-Chain Precursor (11d):



To a dichloromethane solution (190 mL) of the chiral acetal 11a (6.22 g, 19.1 mmol) was added at -78°C trimethylallylsilane (10.9 mL, 68.69 mmol), followed by neat TiCl_4 (2.94 mL, 26.71 mmol). The reaction was stirred at -78°C for 1 hr and then at -40°C for 2 hr. At this point, the reaction was quenched with methanol (15 mL) and diluted with dichloromethane (200 mL). The resulting reaction mixture was washed with 1N HCl (2 x 50 mL), water and brine. The organic layer was dried and conc. in vacuo to give a residue, which was purified by silica gel chromatography (10% EtOAc/Hexanes) to give 5.51 g (78%) of the desired product 11b.

^1H NMR of 11a (CDCl_3): δ 4.73 (m, 1H), 4.21 (m, 1H), 3.86 (m, 1H), 1.75 (m, 1H), 1.60-1.10 (m, 35H), 0.80 (m, 3H). ^1H NMR of 11b (CDCl_3): δ 5.81 (m, 1H), 5.05 (m, 2H), 4.12 (m, 1H), 3.86 (m, 1H), 3.41 (m, 1H), 2.22 (m, 2H), 1.67-1.18 (m, 36H), 0.88 (m, 3H).

To a dichloromethane solution (155 mL) of 11b (8.56 g, 23.3 mmol) was added PCC (10.0 g, 46.5 mmol). The reaction was stirred at rt for 18 hr, and then filtered through a pad of Celite. The filtrates were concentrated in vacuo to give a reddish residue, which was purified by silica gel chromatography (10% EtOAc/Hexanes) to give 8.36 g (80%) of the methyl ketone intermediate (structure not shown). The

intermediate obtained herein (8.36 g, 22.8 mmol) was dissolved in THF (60 mL) and MeOH (30 mL). To this solution was added 7.5 M KOH (15 mL). After stirring 3 hr at rt, the solvent was partially removed. The remaining reaction
5 mixtures were diluted with EtOAc/Et2O (3:1 ratio, 350 mL). The organic layer was washed with water (3 x 50 mL) and brine. The resulting organic layer was dried and conc. in vacuo to give a residue, which was purified by silica gel chromatography (10% EtOAc/Hexanes) to afford 6.22 g (96%) of
10 the desired product 11c as white solids.

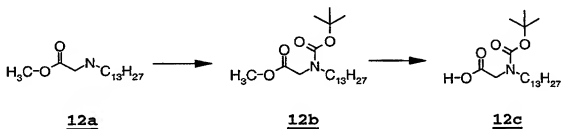
¹H NMR of 11c (CDCl₃): δ 5.75 (m, 1H), 5.06 (m, 2H), 3.56 (m, 1H), 2.23 (m, 1H), 2.07 (m, 1H), 1.75-1.17 (m, 28H), 0.80 (m, 3H).

Carbinol 11c (6.22 g, 22.0 mmol) was dissolved in an
15 aqueous THF solution (5.5 mL water and 55 mL THF). To this solution was added NMO (4.42 g, 33.0 mmol), followed by OsO₄ (280 mg dissolved in THF, 1.10 mmol). The reaction stirred at rt overnight. At this time, sodium bisulfide (4 g) was added. The reaction was stirred for 2 hr, and then diluted
20 with EtOAc (300 mL). The whole mixture was washed with water (2 x 40 mL) and brine. The resulting organic layer was dried and conc. in vacuo to give the corresponding triol intermediate. This material was dissolved in MeOH (200 mL) and water (40 mL). To this solution was added NaIO₄ (10.6 g,

49.5 mmol). After stirring at rt for 1hr, the reaction was filtered through Celite and purified by short column silica gel chromatography (30% EtOAc/Hexanes) to afford ~10 g (>100%) crude beta-hydroxyl aldehyde. The impuried aldehyde thus obtain was dissolved in t-BuOH (100 mL) and cyclohexene (14 mL). To this solution at rt was added an aqueous solution (50 mL) of NaClO₂ (15.97 g, 176 mmol) and KH₂PO₄ (17.8 g, 132 mmol). The reaction was stirred at rt for 6 hr and then quenched at 0°C with 5N HCl to pH=4. The reaction was extracted with 3:1 mix-solvent EtOAc/Et₂O (3 x 250 mL). The organic layer was washed with brine and dried and conc. to provide 7.3 g (>100%) of the crude acid 11d, which was used directly for the coupling reaction.

¹H NMR of 11d (CDCl₃): δ 3.95 (m, 1H), 2.60-2.35 (m, 2H), 1.40-1.10 (m, 28H), 0.82 (m, 3H).

Preparation of Side-Chain Precursor (12c):



A 1 liter round bottom flask was charged with tridecenal (5.00 g, 25.2 mmol) and the HCl salt of Gly-Ome (12.66 g, 100.8 mmol) in 600 ml anhydrous MeOH. To this

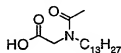
reaction mixture was added NaCNBH_3 (1.787 g, 28.44 mmol) and the reaction was allowed to stir overnight at room temperature. The solids were filtered off and the solvent was removed *in vacuo*. The resulting residue was partitioned
5 between CH_2Cl_2 /saturated aqueous NaHCO_3 . The organic layer was washed 2x NaHCO_3 and dried over MgSO_4 . The drying agent was then filtered off and the solvent was removed *in vacuo*. Purification on a silica gel column eluting with 50% EtOAc/hexanes yielded 2.94 g of a white solid (12a).

10 A 50 ml round bottom flask was charged with the glycine derivative 12a (504.6 mg, 1.86 mmol), triethylamine (224.6 mg, 2.22 mmol) in 10 ml anhydrous THF. To this was added $(\text{BOC})_2\text{O}$ (494.3 mg, 2.26 mmol) in one portion. The reaction was allowed to stir for 18 h at which time the solvent was
15 removed *in vacuo*. The resulting oil was taken up in EtOAc and washed 2x 1N HCl, 1x water, 1x brine and dried over MgSO_4 . The drying agent was then filtered off and the solvent was removed *in vacuo* to yield 463.3 mg of a colorless oil (12b) which was used without purification.

20 A 50 ml round bottom flask containing 5 ml THF was charged with the methyl ester 12b (463.3 mg, 1.25 mmol). To this was added 1.8 ml of a 1N LiOH solution. The resulting reaction mixture was allowed to stir overnight. The reaction was quenched by the addition of 1.8 ml of a 1N HCl

solution. The THF was then removed in vacuo and the resulting aqueous layer was extracted 2x CH₂Cl₂. The organics were then dried over MgSO₄. The drying agent was then filtered off and the solvent was removed in vacuo to yield 246.2 mg of a colorless oil (12c) which was used without purification.

Preparation of Side-Chain Precursor (13a):



13a

10

The methyl ester 12b from above (499.7 mg/1.84 mmol) was dissolved in a 1:1 mixture of acetic anhydride/pyridine in a 50 mL round bottom flask. The reaction was allowed to stir overnight at which time the solvent was removed in vacuo. The resulting oil was taken up in CH₂Cl₂ and washed 2x 1N HCl, 1x water, 1x brine and dried over MgSO₄. The drying agent was then filtered off and the solvent was removed in vacuo to yield 319.9 mg of a colorless oil (13a) which was used without purification.

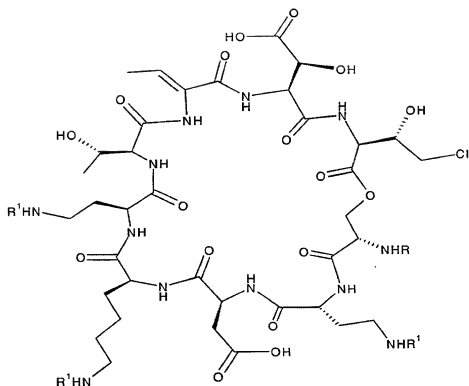
20

General Preparation for Glycine side-chain precursors (14-a):

For example the following procedure describes the synthesis of tridecanoyl-glycine-acid from Fmoc-glycine-wang resin and tridecanoic acid.

In a 100mL double-ended glass fritted reaction tube,
5 Fmoc-glycine-wang resin (10g, 4.4 mmol) was added to 50 mL of 30% Piperdine/DMF. The reaction was shaken for 20 minutes, and was washed 3x with DMF, 3x with isopropanol, and 3x with DMF. To the resin was added a solution of tridecanoic acid (4.708g, 22 mmol) in 50 mL DMF. To this
10 mixture was added HOBt (2.97 g, 22 mmol) and DIC (2.77 g, 22mmol). The reaction vessel was put on a shaker overnight. The resin was then washed 3x with DMF, 3x with dichloromethane, 3x with MeOH, 3x with THF, and 3x with dichloromethane. The resin beads were dried in a vacuum
15 oven for 1 hour. To the resin was added 100 mL of 95% TFA/H₂O. The reaction was shaken for 1.5 hours, and the non-resin product was washed with TFA and collected. The product was dried in a vacuum oven to a white residue, and azeotroped with Toluene to yield tridecanoyl-glycine-acid
20 (1.14 g, 95%) ¹HNMR (THF) 0.82-0.92 (t, J= 7.2, 3H), 1.2-1.4 (s, 18H), 1.52-1.62 (m, 2H), 2.10-2.17 (t, J=7.2 , 2H), 3.83-3.89 (d, J=7.1 , 2H), 7.04-7.17 (s, 1H), 10.8-10.9 (s, 1H).

The following structure II will be used to describe the
25 products observed in Examples 1 through 17.



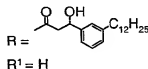
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1991	100	100	100	100
1992	100	100	100	100
1993	100	100	100	100
1994	100	100	100	100
1995	100	100	100	100
1996	100	100	100	100
1997	100	100	100	100
1998	100	100	100	100
1999	100	100	100	100
2000	100	100	100	100
2001	100	100	100	100
2002	100	100	100	100
2003	100	100	100	100
2004	100	100	100	100
2005	100	100	100	100
2006	100	100	100	100
2007	100	100	100	100
2008	100	100	100	100
2009	100	100	100	100
2010	100	100	100	100
2011	100	100	100	100
2012	100	100	100	100
2013	100	100	100	100
2014	100	100	100	100
2015	100	100	100	100
2016	100	100	100	100
2017	100	100	100	100
2018	100	100	100	100
2019	100	100	100	100
2020	100	100	100	100

Category	Sub-category	Value	Unit
Energy	Electricity	1000	kWh
	Gas	500	m ³
Water	Supply	100	m ³
	Wastewater	100	m ³
Waste	Recycling	50	kg
	Landfill	50	kg
Materials	Concrete	100	m ³
	Steel	100	kg

[illegible]

consumption of CBZ-protected pseudomycin nucleus (Z-PSN) after a period of 5 hours. The solvent was removed in vacuo and the resulting residue was taken up in 1:1 ACN/H₂O and purified via preparatory HPLC. This yielded 2 major peaks whose mass spectral data suggests that these peaks correspond to the 2 diastereomers 1-1 (88.3 mg) and 1-2 (166.3mg).

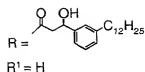
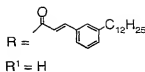
Deprotection of Diastereomer 1-1:



1-3

A 50 ml round bottom flask was charged with 10 ml MeOH /1 ml glacial AcOH and diastereomer 1-1 (82.0 mg, 0.048 mmol). After degassing 89.1 mg of 10% Pd/C was added to the reaction mixture and subjected to 1 atm H₂ for 30 minutes. Removal of the catalyst via filtration and purification via preparatory HPLC and subsequent lyophilization provided 21.7 mg of Compound 1-3 (R¹ = H). MS (Ionspray) calcd for C₅₃H₉₄ClN₁₂O₁₉ (M+H)⁺ 1297.64, found 1297.8.

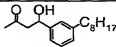
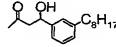
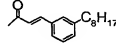
Deprotection of Diastereomer 1-2:

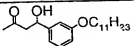
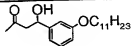
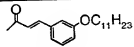
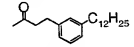
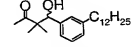
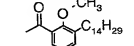
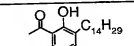
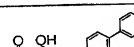
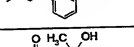
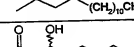
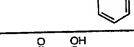
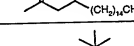
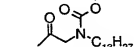
**1-4****1-5**

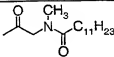
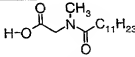
Diastereomer **1-2** (152.8 mg, 0.089mmol) was subjected to hydrogenolysis as described for diastereomer **1-1** using 152.8 mg of 10% Pd/C for 30 min. HPLC indicated consumption of starting material and formation of two product peaks which, after preparatory HPLC and lyophilization, were found to be Compound **1-4** (18.0 mg) and Compound **1-5** (11.3 mg). Compound **1-4**: MS (Ionspray) calcd for $\text{C}_{58}\text{H}_{94}\text{ClN}_{12}\text{O}_{19}$ $(\text{M}+\text{H})^+$ 1297.89, found 1297.8. Compound **1-5**: MS (Ionspray) calcd for $\text{C}_{58}\text{H}_{92}\text{ClN}_{12}\text{O}_{18}$ $(\text{M}+\text{H})^+$ 1279.63, found 1281.7.

Each of the compounds listed in Table 1 below was synthesized using the same acylation and deprotection procedures as described above using the indicated side-chain precursor. For each compound listed in Table 1, R^1 is a hydrogen.

Table 1

Example No.	R =	Side-Chain Precursor
2-1		2c
2-2		2c
2-3		2c

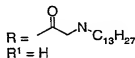
3-1		3b
3-2		3b
3-3		3b
4-1		4b
5-1		5a
6-1		6a
7-1		7a
8-1		8b
9-1		9a
10-1		10e
11-1		11d
12-1		12c
13-1		13a

14-1*		 (available from Sigma)
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- *Although this compound and the compound where the N-methyl group is absent shows little activity, Compounds where the alkyl chain is increased progressively from C11 to C15 show significant increased activity. Side chains of this class may be prepared using the preparation described in preparation 14-a.

Example 15

Synthesis of Compound 15-1:



15-1

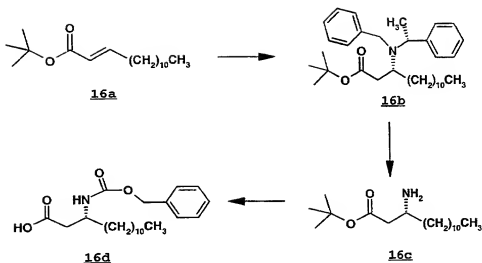
To a 50 ml round bottom flask was added 31.3 mg (0.0237 mmol) of Compound 12-1 and 5 ml TFA (precooled to 0 °C).

- The reaction mixture was allowed to stir at this temperature for a period of 15 min at which time the TFA was removed *in vacuo*. The residue was then taken up in water and lyophilized to yield 24.3 mg of Compound 15-1. No further purification was necessary.

Example 16

- Example 16 illustrates the attachment of a Beta-amino substituted side-chain.

Preparation of Compounds 16a, 16b, 16c and 16d:



A solution of t-butoxycarbonylmethylenetriphenyl-phosphorane (5.0 g, 13.3 mmol) and dodecyl aldehyde (2.2 ml, 10 mmol) in toluene (50 ml) was refluxed for 1 hr 20 min. The solution was filtered through a plug of silica to remove the phosphorus reagents and then reduced in vacuo to give a crude oil. The oil was purified over a silica column by elution with 2% ethyl acetate in hexane to give 2.36 g (84% yield) of compound **16a**. MS- 283.4 (M+1) NMR consistent with structure.

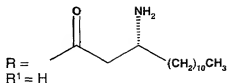
Butyl lithium (1.6 M in hexane) (1.19 ml, 1.9 mmol) was added slowly to a solution of (R)-benzylmethyl benzylamine (0.42ml, 2.0 mmol) in THF (5 ml) cooled to -78°C . A THF solution of **16a** (500 mg, 1.77 mmol) was then added dropwise. The mixture was stirred at -78°C for 1 hr. The reaction mixture was then poured into sat. NH_4Cl solution and extracted with ether (2x). The ether solution was dried over MgSO_4 and reduced in vacuo to give 0.95 g of **16b** as a

crude oil which was carried to the next step without purification.

An ethanol (25 ml) solution of 16b (0.95 g) and Pd(OH)₂/C (0.47 g) was put under 60 psi of H₂ at 55 °C for 18
5 hr. The suspension was filtered and then reduced in vacuo to give 280 mg (53% yield) of crude 16c which was carried directly to the next step.

Compound 16c (280 mg, 0.93 mmol) and N-benzyloxy-carbonyloxysuccinimide (274 mg, 1.1 mmol) were mixed in THF
10 (10 ml) and stirred overnight. The solvent was removed in vacuo and the product oil was purified by column chromatography over silica using 5% ethyl acetate in hexane as the eluant to give 268 mg (66% yield) of t-butyl ester of 16d. NMR was appropriate for the expected structure. The
15 ester (97 mg, 0.224 mmol) was dissolved in trifluoroacetic acid (2 ml) at 0 °C for 0.5 hr to remove the t-butyl ester (16d) with a quantitative yield.

Synthesis of Compound 16-1:



Compound **16d** was dissolved in DMF (2 ml).

Hydroxybenzotriazole (36.4 mg, 0.269 mmol) and 1-[3-

5 (dimethylamino)propyl]-3-ethylcarbodiimide hydrochloride
(47.1 mg, 0.246 mmol) were added and the solution was
stirred 18 hr. The CBZ protected pseudomycin nucleus (Z-
PSN) (277 mg, 0.185 mmol) was added and the reaction was
stirred for an additional 18 hr. The reaction product was
10 purified by HPLC and lyophilization gave 136.3 mg (42%
yield) of the acylated CBZ protected pseudomycin derivative
as a white solid. MS- 1743 (M) and the NMR was consistent
with the proposed structure.

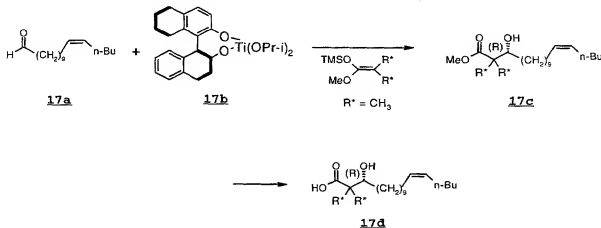
A methanol (10 ml) and acetic acid (1.5 ml) solution of the acylated compound (130 mg, 0.0746 mmol) with 10% Pd/C (120 mg) was put under a balloon of hydrogen for 20 min. The solution was filtered and purified by preparative HPLC. Lyophilization gave 23 mg (18% yield) of the trifluoroacetic acid salt of **16-1** as a white solid. MS- 1206.8 (M) and the NMR was consistent with the proposed structure. Compound **16-1** showed little or no activity against *Candida Albicans*.

and *Cryptococcus neoformans* which is a significant reduction in activity as compared to the β -hydroxy analog.

Example 17

Example 17 illustrates the attachment of a chiral side-chain.

Preparation of side chain precursor 17d:



To a THF solution of (S)-binaphthol (240 mg, 0.84 mmol) was added 4A molecular sieves (4 g), followed by addition of neat Ti(OPr-i)₄ (0.25 mL, 0.84 mmol). The reaction mixture turned red immediately and remained to be red. The chiral catalyst 17b thus prepared was used for the subsequent reaction.

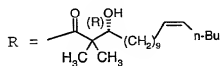
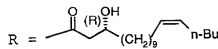
To a freshly prepared THF solution (4 mL) of (S)-binaphthol-Ti catalyst 17b (0.42 mmol) was added at -78°C a THF solution containing trimethylsilyldimethylketene acetal (0.43 mL, 2.1 mmol) and the unsaturated aldehyde 17a (500 mg, 2.1 mmol) over 15 min. The reaction was stirred at -78°C

for 1 hr and then at rt overnight. At this point, the reaction was quenched with saturated NaHCO_3 solution and extracted with EtOAc (100 mL). The organic layer was washed with NaHCO_3 , brine, and dried with anhydrous MgSO_4 . Upon
5 filtration and conc. in vacuo. The residue was purified with silica gel chromatography (10% EtOAc/Hexanes) to give 257 mg (36%) of the desired product 17c.

^1H NMR of 17c (CDCl_3): δ 5.29 (m, 2H), 3.65 (s, 3H), 3.53 (t, $J = 7.3$ Hz, 1H), 2.33 (d, $J = 6.3$ Hz, 1H, 3'-OH), 1.97 (m, 4H), 1.65-1.22 (m, 20H), 1.13 (s, 3H), 1.12 (s, 3H), 0.84 (m, 3H).

A THF solution (5 mL) of 17c (259 mg, 0.76 mmol) was treated with an aqueous solution of NaOH (0.30 mL, 5N, 1.52 mmol). The reaction was heated overnight at 50°C . The
15 reaction mixture was cooled to 0°C and acidified to pH = 3 using 5N HCl. The reaction was then extracted with EtOAc (75 mL). The organic layer was washed with water and brine. The organic layer thus obtained was dried and conc. in vacuo to afford 222 mg (90%) of the crude acid 17d, which was used
20 directly for side chain coupling reaction.

^1H NMR of 17d (CDCl_3): δ 5.28 (m, 2H), 3.56 (m, 1H), 1.95 (m, 4H), 1.60-1.10 (m, 26H), 0.83 (m, 3H).

Preparation of 17-1 and 17-2:R¹ = H17-1R¹ = H17-2

A THF solution (7 mL) of the crude acid **17d** (240 mg, 0.74 mmol) was treated with HOBt (90.5 mg, 0.67 mmol) and EDCI (128 mg, 0.67 mmol) at rt. After stirring for 1.5 hr, DMAP (41 mg, 0.33 mmol) was added. After stirring for another 2 hr, a DMF (4 mL) of CBZ-protected pseudomycin nucleus (614 mg, 0.44 mmol) was added and the reaction was stirred at rt overnight. The reaction mixture was purified with preparative HPLC to give, after lyophilization, (160 mg, 21%) of the desired product.

An acetonitrile solution (2 mL) of CBZ-protected compound (53.4 mg, 0.032 mmol) was treated with TMSI (77 mg, 0.38 mmol) at 0°C. After 100 min, the reaction was quenched with 1:1 CH₃CN/H₂O. The resulting reaction mixture was purified by reverse phase preparative HPLC to give 25.8 mg (65%) of the desired final product 17-1.

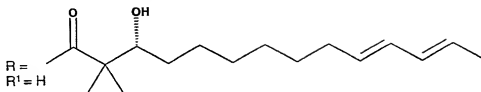
Compound 17-2 is made using the same procedures described above using the appropriate starting materials where R* is a hydrogen.

2H), 1.80-1.60 (m, 3H), 1.60-1.40 (m, 3H), 1.38-1.10 (m, 15H). ^1H NMR of 18b (CDCl_3): δ 5.98-5.90 (m, 2H), 5.55-5.47 (m, 2H), 4.08 (m, 1H), 3.86 (m, 1H), 3.61 (s, 3H), 3.50 (m, 1H), 2.00-1.96 (m, 2H), 1.74-1.63 (m, 3H), 1.50-1.00 (m, 5 24H).

To a dichloromethane solution (22 mL) of 18b (837 mg, 2.27 mmol) was added PCC (0.98 g, 4.54 mmol). The reaction was stirred at rt for 18 hr, and then filtered through a pad of Celite. The filtrates were concentrated in vacuo to give 10 a reddish residue, which was purified by silica gel chromatography (20% EtOAc/Hexanes) to give 441 mg (53%) of the desired methyl ketone 18c.

^1H NMR of 18c (CDCl_3): δ 5.92-5.87 (m, 2H), 5.48-5.43 (m, 2H), 3.88 (m, 1H), 3.56 (s, 3H), 3.49 (m, 1H), 2.59 (dd, J = 15 6.4, 14.7 Hz, 1H), 2.29 (dd, J = 5.9, 15.2 Hz, 1H), 2.06 (s, 3H), 1.96 (m, 2H), 1.63 (m, 2H), 1.40-0.90 (m, 20H).

To a THF (4 mL) and methane (2 mL) solution of 18c (440 mg, 1.20 mmol) was added at rt 7.5M KOH (1 mL). After stirring at rt for 4 h, the reaction was acidified with 1N 20 HCl to pH = 3. The reaction mixture was extracted with EtOAc (3 x 30 mL). The combined extracts were washed with water (2 x 10 mL) and brine. The organic layer was dried and conc. in vacuo to give 388 mg (>100%) of the crude acid 18d, which was used directly for the side chain coupling reaction.

Preparation of Compound 18-1:18-1

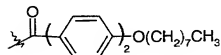
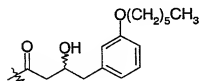
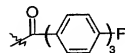
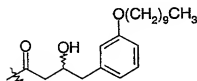
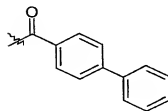
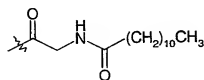
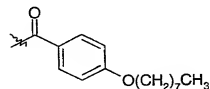
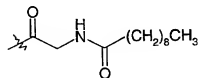
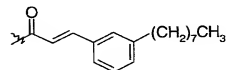
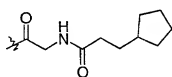
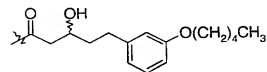
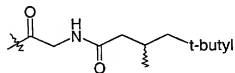
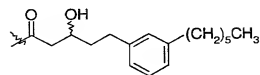
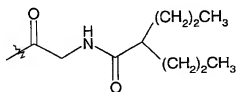
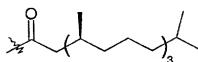
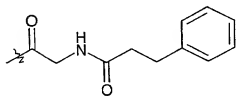
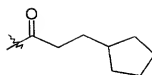
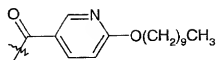
To a THF solution (10 mL) of the crude acid 18d (~1.66
 5 mmol) was added HOBT (244 mg, 1.66 mmol) and EDCI (318 mg,
 1.66 mmol). After stirring at rt for 5 hr, a DMF solution (5
 mL) of Alloc-protected pseudomycin nucleus (614 mg, 0.50
 mmol) was added. After stirring at rt for a few days, DMAP
 (61 mg, 0.50 mmol) was added to the reaction mixture. After
 10 stirring for additional 12 hr, the reaction mixture was
 purified by reverse phase HPLC to afford, after
 lyophilization, 225 mg (30%) of alloc-protected acylated
 derivative.

To a degassed THF (20 mL) and HOAc (1 mL) solution of
 15 alloc-protected acylated derivative (240 mg, 0.16 mmol) was
 added $\text{PdCl}_2(\text{PPh}_3)_2$ (23 mg, 0.032 mmol) and Bu_3SnH (0.87 mL,
 3.23 mmol) at rt. After 1.5 hr, the reaction mixture was
 purified by preparative HPLC to afford 33 mg (17%) of
 Compound 18-1.

20 In addition to the compounds listed in the Examples
 above, the following N-acyl derivatives were also made which
 showed limited activity or non-significant activity.

Even though these compounds may not be as useful as antifungal agents, they provide valuable insight into the design of compounds having optimal activity.

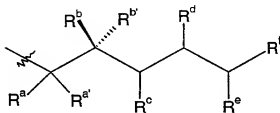
1. A compound of the formula (I) as defined in claim 1, wherein the substituent R is a hydrogen atom.



Unless indicated otherwise, each of the compounds listed in the Examples showed measurable activity against *Candida Albicans*, *Cryptococcus neoformans*, *Aspergillus Fumigatus*, *Candida Parapsilosis*, or *Histoplasma capsulatum*.

- 5 However, the following basic trends in activity were observed based on the compounds synthesized. The stereochemistry of the β -hydroxy group is preferably R. Longer alkyl chain lengths (i.e., C₁₂-C₂₀) tend to have higher activities than shorter alkyl chains (e.g., < C₁₁)
- 10 regardless of stereochemistry or unsaturation levels. Removal of the β -hydroxy group, α,α -disubstitution, lower alkyl chain lengths in both alkyl and alkoxy substituents, extreme rigidity, and increased branching in the chain all
- 15 tended to have lower activity than the longer flexible chains.

Consequently, alkyl side-chains represented by the following structure are preferred for antifungal treatment:



where

- 20 R^a and $R^{a'}$ are independently hydrogen or methyl, or either R^a or $R^{a'}$ is alkyl amino, taken together with R^b

or R^{b'} forms a six-membered cycloalkyl ring, a six-membered aromatic ring or a double bond, or taken together with R^c forms a six-membered aromatic ring;

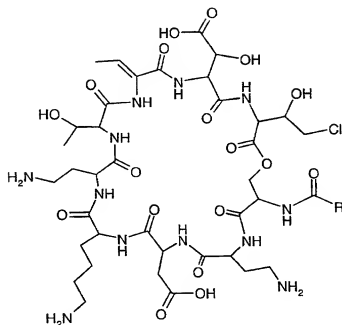
5 R^b and R^{b'} are independently hydrogen or methyl, and either R^b or R^{b'} is hydroxy provided that R^{b'} is not hydroxy when R^a, R^b, R^d, R^e are hydrogen, R^c is hydrogen and R^f is *n*-hexyl, *n*-octyl or *n*-decyl, or R^a, R^b, R^d, R^e are hydrogen, R^c is hydroxy and R^f is *n*-octyl, *n*-nonyl, or *n*-decyl;

10 R^c is hydrogen, hydroxy, C₁-C₄ alkoxy, hydroxyalkoxy, or taken together with R^e forms a 6-membered aromatic ring or C₅-C₆ cycloalkyl ring;

15 R^e is hydrogen, or taken together with R^f is a six-membered aromatic ring, C₅-C₁₄ alkoxy substituted six-membered aromatic ring, or C₅-C₁₄ alkyl substituted six-membered aromatic ring, and

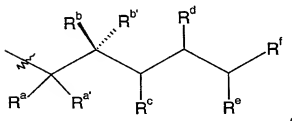
 R^f is C₈-C₁₄ alkyl, or C₅-C₁₁ alkoxy.

20



10

1000



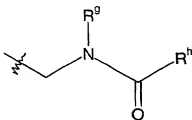
R^b and $R^{b'}$ are independently hydrogen, halogen, or methyl, or either R^b or $R^{b'}$ is amino, alkylamino, α -acetoacetate, methoxy, or hydroxy provided that $R^{b'}$ is not hydroxy when R^a , R^b , R^d , R^e are hydrogen, R^c is hydrogen and R^f is *n*-hexyl, *n*-octyl or *n*-decyl, or R^a , R^b , R^d , R^e are hydrogen, R^c is hydroxy and R^f is *n*-octyl, *n*-nonyl, or *n*-decyl;

R^c is hydrogen, hydroxy, C_1 - C_4 alkoxy, hydroxyalkoxy, or taken together with R^e forms a 6-membered aromatic ring or C_5 - C_6 cycloalkyl ring;

R^e is hydrogen, or taken together with R^f is a six-membered aromatic ring, C_5 - C_{14} alkoxy substituted six-membered aromatic ring, or C_5 - C_{14} alkyl substituted six-membered aromatic ring, and

R^f is C_8 - C_{18} alkyl, C_5 - C_{11} alkoxy, or biphenyl; or

R is

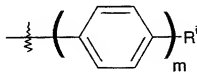


where

R^g is hydrogen, or C_1 - C_{13} alkyl, and

R^h is C_1 - C_{15} alkyl, C_4 - C_{15} alkoxy, (C_1 - C_{10} alkyl)phenyl, $-(CH_2)_n$ -aryl, or $-(CH_2)_n$ -(C_5 - C_6 cycloalkyl), where $n = 1-2$; or

R is

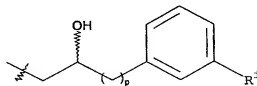


5

where

R^i is a hydrogen, halogen, or C_5 - C_8 alkoxy, and m is 1, 2 or 3;

R is

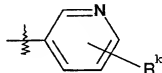


10

where

R^j is C_5 - C_{14} alkoxy or C_5 - C_{14} alkyl, and $p = 0, 1$ or 2;

R is



15

where

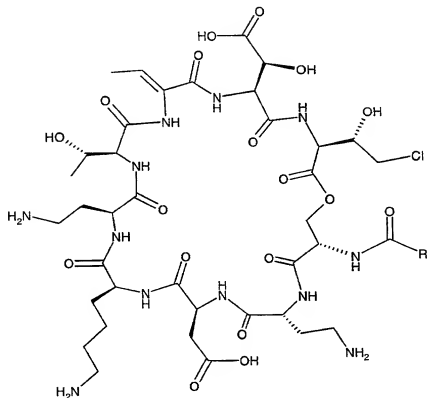
R^k is C_5 - C_{14} alkoxy; or

R is $-(CH_2)-NR^m-(C_{13}-C_{18} \text{ alkyl})$, where R^m is H, $-CH_3$ or

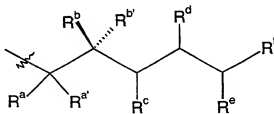
$$-\text{C}(\text{O})\text{CH}_3; \text{ and}$$

pharmaceutically acceptable salts and solvates thereof.

2. The compound of Claim 1 wherein structure I has
5 the following stereochemistry



3. The compound of Claim 1 wherein R is



10

where

5 R^a and $R^{a'}$ are independently hydrogen or methyl, or either R^a or $R^{a'}$ is alkyl amino, taken together with R^b or $R^{b'}$ forms a six-membered cycloalkyl ring, a six-membered aromatic ring or a double bond, or taken together with R^c forms a six-membered aromatic ring;

10 R^b and $R^{b'}$ are independently hydrogen, halogen, or methyl, or either R^b or $R^{b'}$ is amino, alkylamino, α -acetoacetate, methoxy, or hydroxy provided that $R^{b'}$ is not hydroxy when R^a , R^b , R^d , R^e are hydrogen, R^c is hydrogen and R^f is *n*-hexyl, *n*-octyl or *n*-decyl, or R^a , R^b , R^d , R^e are hydrogen, R^c is hydroxy and R^f is *n*-octyl, *n*-nonyl, or *n*-decyl;

15 R^c is hydrogen, hydroxy, C_1 - C_4 alkoxy, hydroxyalkoxy, or taken together with R^e forms a 6-membered aromatic ring or C_5 - C_6 cycloalkyl ring;

20 R^e is hydrogen, or taken together with R^f is a six-membered aromatic ring, C_5 - C_{14} alkoxy substituted six-membered aromatic ring, or C_5 - C_{14} alkyl substituted six-membered aromatic ring, and

R^f is C_8 - C_{18} alkyl, C_5 - C_{11} alkoxy, or biphenyl.

4. The compound of Claim 3 wherein $R^{b'}$ is hydroxy provided that R^c is not hydrogen when R^a , R^b , R^d , R^e are

hydrogen and R^f is *n*-hexyl, *n*-octyl or *n*-decyl, or R^c is not hydroxy when R^f is *n*-octyl, *n*-nonyl, or *n*-decyl.

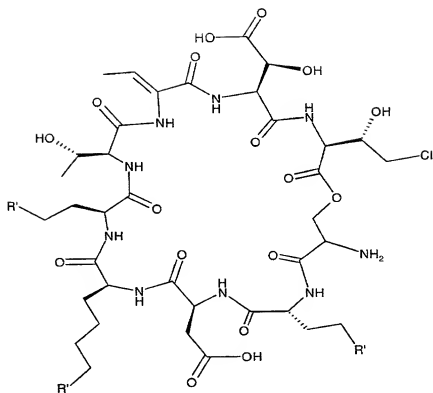
5 5. The use of a compound as claimed in any one of the preceding claims in the preparation of a medicament for use in combating either systemic fungal infections or fungal skin infections.

10 6. A pharmaceutical formulation comprising a pseudomycin compound of Claim 2 and a pharmaceutically acceptable carrier.

15 7. A method for treating an antifungal infection in an animal in need thereof, comprising the steps of administering to said animal a pseudomycin compound of Claim 2.

20 8. A process for producing a pseudomycin nucleus comprising the steps of providing a pseudomycin compound having an N-acyl alkyl side-chain containing at least one gamma or delta hydroxy group and reacting said pseudomycin compound with an acid to produce said pseudomycin nucleus.

25 9. The process of Claim 8 wherein said pseudomycin nucleus is represented by structure I-A



2. 40

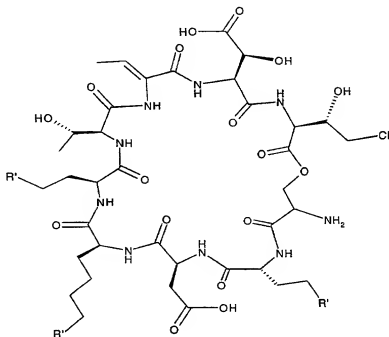
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1992	1,200	1.2
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1994	1,400	1.4
1995	1,500	1.5
1996	1,600	1.6
1997	1,700	1.7
1998	1,800	1.8
1999	1,900	1.9
2000	2,000	2.0
2001	2,100	2.1
2002	2,200	2.2
2003	2,300	2.3
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2005	2,500	2.5
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2009	2,900	2.9
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2016	3,600	3.6
2017	3,700	3.7
2018	3,800	3.8
2019	3,900	3.9
2020	4,000	4.0

$$I_{\text{max}} = I_0$$

12. The process of Claim 11 wherein said acid is trifluoroacetic acid.

13. A pseudomycin nucleus prepared by the process of Claim 8.

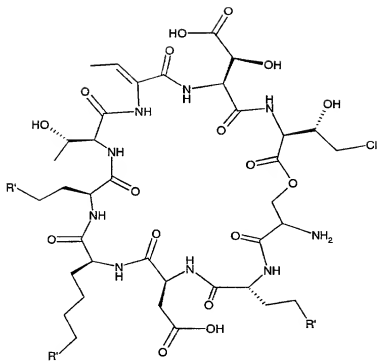
14. The pseudomycin nucleus of Claim 13 wherein said nucleus is represented by structure I-A



I-A

wherein R' is -NH₂ or -NHp-Pg where Pg is an amino protecting group and p is 0 or 1.

15. A pseudomycin nucleus represented by structure I-A



I-A

wherein R' is -NH₂ or -NHp-Pg where Pg is an amino protecting group and p is 0 or 1.

Please type a plus sign (+) inside this box ☐

PTO/SB/01 (8-96) (MODIFIED)

Approved for use through 9/30/98. OMB 0651-0032
Patent and Trademark Office: U.S. DEPARTMENT OF COMMERCE

DECLARATION FOR UTILITY OR DESIGN PATENT APPLICATION

- ☒ Declaration Submitted with Initial Filing
☐ Declaration Submitted after Initial Filing

Attorney Docket Number	X-11260
First Named Inventor	Matthew David Belvo
COMPLETE IF KNOWN	
Application Number	
Filing Date	
Group Art Unit	
Examiner Name	

As a below named inventor, I hereby declare that:

My residence, post office address, and citizenship are as stated below next to my name.

I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled:

PSEUDOMYCIN N-ACYL SIDE-CHAIN ANALOGS

the specification of which
☐ is attached hereto
OR

☒ was filed on
(MM/DD/YYYY)

08 June 2000

as United States Application Number or PCT International

Application
Number

PCT/US00/15017

and was amended on
(MM/DD/YYYY)

(If applicable).

I hereby state that I have reviewed and understand the contents of the above-identified specification, including the claims, as amended by any amendment specifically referred to above.

I acknowledge the duty to disclose information which is material to patentability as defined in Title 37 Code of Federal Regulations, § 1.56.

I hereby claim foreign priority benefits under Title 35, United States Code § 119(a)-(d) or § 365(b) of any foreign application(s) for patent or inventor's certificate, or § 365(a) of any PCT international application which designated at least one country other than the United States of America, listed below and have also identified below, by checking the box, any foreign application for patent or inventor's certificate, or of any PCT international application having a filing date before that of the application on which priority is claimed.

Prior Foreign Application Number(s)	Country	Foreign Filing Date (MM/DD/YYYY)	Priority Not Claimed	Certified Copy Attached	
				YES	NO
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			<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
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			<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
			<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
			<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>

☐ Additional foreign application numbers are listed on a supplemental priority sheet attached hereto:

I hereby claim the benefit under Title 35, United States Code § 119(e) of any United States provisional application(s) listed below.

Application Number(s)	Filing Date (MM/DD/YYYY)	<input type="checkbox"/> Additional provisional application numbers are listed on a supplemental priority sheet attached hereto.
60/143,989	15 July 1999	

Please type a plus sign (+) inside this box

PTO/SB/01 (8-96) (MODIFIED)

Approved for use through 9/30/98. OMB 0651-0032
Patent and Trademark Office: U.S. DEPARTMENT OF COMMERCE**DECLARATION**

I hereby claim the benefit under Title 35, United States Code §120 of any United States application(s), or § 365(c) of any PCT international application designating the United States of America, listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States or PCT international application in the manner provided by the first paragraph of Title 35, United States Code § 112, I acknowledge the duty to disclose information which is material to patentability as defined in Title 37, Code of Federal Regulations § 1.56 which became available between the filing date of the prior application and the national or PCT international filing date of this application.

U.S. Parent Application Number	PCT Parent Number	Parent Filing Date (MM/DD/YYYY)	Parent Patent Number (if applicable)

☐ Additional U.S. or PCT international application numbers are listed on a supplemental priority sheet attached hereto.

As a named inventor, I hereby appoint the following registered practitioner(s) to prosecute this application and to transact all business in the Patent and Trademark Office connected therewith:

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Gerald P. Kehler	43,707

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Edward Prein	37,212
Grant E. Reed	41,264
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Michael J. Sayles	32,295
Robert L. Sharp	45,609
David M. Siemerick	40,187
Mark J. Stewart	43,936
Robert C. Titus	40,206
Robert C. Tucker	45,165
Tina M. Tucker	47,145
MacChern Vomdran-Jones	36,711
Gilbert T. Voy	43,972
Thomas D. Webster	39,872
Lawrence T. Welch	29,487
Alexander Wilson	45,762
Dan L. Wood	48,613

☐ Additional registered practitioner(s) named on a supplemental sheet attached hereto.

Direct all correspondence to:

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 Address **ATTN: TINA M. TUCKER**
 Address **LILLY CORPORATE CENTER/DC1104**
 City **INDIANAPOLIS** State **INDIANA** ZIP **46285**
 Country _____ Telephone **(317) 277-3537** Fax **(317) 276-3861**

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Name of Sole or First Inventor:

☐ A Petition has been filed for this unsigned inventor

Given Name **Matthew** Middle Name **David** Family Name **Belvo** Suffix e.g. Jr. _____
 Inventor's Signature *Matthew David Belvo* Date **11-28-01**
 Residence: City **Greenfield** State **IN** Country **USA** Citizenship **USA**
 Address **24 Greenbrook Court**
 Post Office Address **SAME AS ABOVE**
 City **Greenfield** State **IN** Zip **46140** Country **USA**

☒ Additional Inventors are being named on supplement sheet(s) attached hereto.

Please type a plus sign (+) inside this box

+

PTO/SB/01 (8-95) (MODIFIED)

Approved for use through 9/30/98. OMB 0651-0032
Patent and Trademark Office: U.S. DEPARTMENT OF COMMERCE

DECLARATION

Name of Additional Joint Inventor, if any:				<input type="checkbox"/> A Petition has been filed for this unsigned inventor			
Given Name	Shu	Middle Name	Hui	Family Name	Chen	Suffix e.g. Jr.	
Inventor's Signature	<i>Chen Shu Hui</i>				Date	11/30/00	
Residence: City	Carmel	State	IN	Country	USA	Citizenship	USA
Address	13256 Snow Owl Drive IN						
Post Office Address	SAME AS ABOVE						
City	Carmel	State	IN	Zip	46033	Country	USA

Name of Additional Joint Inventor, if any:				<input type="checkbox"/> A Petition has been filed for this unsigned inventor			
Given Name	Christopher	Middle Name	William	Family Name	Doecke	Suffix e.g. Jr.	
Inventor's Signature	<i>Christopher William Doecke</i>				Date	12/9/00	
Residence: City	Indianapolis	State	IN	Country	USA	Citizenship	USA AU <i>add</i>
Post Office Address	7515 East 80 th Street IN						
Post Office Address	SAME AS ABOVE						
City	Indianapolis	State	IN	Zip	46256	Country	USA

Name of Additional Joint Inventor, if any:				<input type="checkbox"/> A Petition has been filed for this unsigned inventor			
Given Name	Sarah	Middle Name	Lynne	Family Name	Hellman	Suffix e.g. Jr.	
Inventor's Signature	<i>Sarah Lynne Hellman</i>				Date	11/21/01	
Residence: City	Indianapolis	State	IN	Country	USA	Citizenship	USA
Post Office Address	7009 Ringtail Court IN						
Post Office Address	SAME AS ABOVE						
City	Indianapolis	State	IN	Zip	46254	Country	USA

Name of Additional Joint Inventor, if any:				<input type="checkbox"/> A Petition has been filed for this unsigned inventor			
Given Name	James	Middle Name	Andrew	Family Name	Jamison	Suffix e.g. Jr.	
Inventor's Signature	<i>James Andrew Jamison</i>				Date	11/28/01	
Residence: City	Indianapolis	State	IN	Country	USA	Citizenship	USA
Post Office Address	7009 Ringtail Court IN						
Post Office Address	SAME AS ABOVE						
City	Indianapolis	State	IN	Zip	46254	Country	USA

Please type a plus sign (+) inside this box ☐

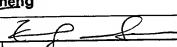
PTO/SB/01 (8-96) (MODIFIED)

Approved for use through 9/30/98, OMB 0651-0032
Patent and Trademark Office: U.S. DEPARTMENT OF COMMERCE

DECLARATION

Name of Additional Joint Inventor, if any:		<input type="checkbox"/> A Petition has been filed for this unsigned inventor					
Given Name	Lawrence	Middle Name	Edward	Family Name	Patterson	Suffix e.g. Jr.	
Inventor's Signature							Date
Residence: City	Indianapolis	State	IN	Country	USA	Citizenship	USA
Address	3839 East 42 nd Street						
Post Office Address	SAME AS ABOVE						
City	Indianapolis	State	IN	Zip	46226	Country	USA

Name of Additional Joint Inventor, if any:		<input type="checkbox"/> A Petition has been filed for this unsigned inventor					
Given Name	Michael	Middle Name	John	Family Name	Rodriguez	Suffix e.g. Jr.	
Inventor's Signature							Date
Residence: City	Indianapolis	State	IN	Country	USA	Citizenship	USA
Post Office Address	7649 Gordonshire Court						
Post Office Address	SAME AS ABOVE						
City	Indianapolis	State	IN	Zip	46278	Country	USA

Name of Additional Joint Inventor, if any:		<input type="checkbox"/> A Petition has been filed for this unsigned inventor					
Given Name	Xicheng	Middle Name	David	Family Name	Sun	Suffix e.g. Jr.	
Inventor's Signature							Date 11/30/2001
Residence: City	Superior	State	CO	Country	USA	Citizenship	China
Post Office Address	923 Grays Peak Drive						
Post Office Address	SAME AS ABOVE						
City	Superior	State	CO	Zip	80027	Country	USA

Name of Additional Joint Inventor, if any:		<input type="checkbox"/> A Petition has been filed for this unsigned inventor					
Given Name	William	Middle Name	Wilson	Family Name	Turner	Suffix e.g. Jr.	
Inventor's Signature							Date
Residence: City	Bloomington	State	IN	Country	USA	Citizenship	USA
Post Office Address	4000 Saratoga Drive						
Post Office Address	SAME AS ABOVE						
City	Bloomington	State	IN	Zip	47408	Country	USA

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Patent and Trademark Office: U.S. DEPARTMENT OF COMMERCE

DECLARATION

Name of Additional Joint Inventor, if any:		<input type="checkbox"/> A Petition has been filed for this unsigned inventor			
Given Name	Lawrence	Middle Name	Edward	Family Name	Patterson
Inventor's Signature	<i>Lawrence Edward Patterson</i>				Suffix e.g. Jr.
Residence: City	Indianapolis	State	IN	Country	USA
Address	3839 East 42 nd Street				
Post Office Address	SAME AS ABOVE				
City	Indianapolis	State	IN	Zip	46226
		Country	USA		

Name of Additional Joint Inventor, if any:		<input type="checkbox"/> A Petition has been filed for this unsigned inventor			
Given Name	Michael	Middle Name	John	Family Name	Rodriguez
Inventor's Signature	<i>Michael John Rodriguez</i>				Suffix e.g. Jr.
Residence: City	Indianapolis	State	IN	Country	USA
Post Office Address	7649 Gordonshire Court				
Post Office Address	SAME AS ABOVE				
City	Indianapolis	State	IN	Zip	46278
		Country	USA		

Name of Additional Joint Inventor, if any:		<input type="checkbox"/> A Petition has been filed for this unsigned inventor			
Given Name	Xicheng	Middle Name	David	Family Name	Sun
Inventor's Signature					Suffix e.g. Jr.
Residence: City	Superior	State	CO	Country	USA
Post Office Address	923 Grays Peak Drive				
Post Office Address	SAME AS ABOVE				
City	Superior	State	CO	Zip	80027
		Country	USA		

Name of Additional Joint Inventor, if any:		<input type="checkbox"/> A Petition has been filed for this unsigned inventor			
Given Name	William	Middle Name	Wilson	Family Name	Turner
Inventor's Signature	<i>William Wilson Turner</i>				Suffix e.g. Jr.
Residence: City	Bloomington	State	IN	Country	USA
Post Office Address	4000 Saratoga Drive				
Post Office Address	SAME AS ABOVE				
City	Bloomington	State	IN	Zip	47408
		Country	USA		

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DECLARATION

Name of Additional Joint Inventor, if any:				<input type="checkbox"/> A Petition has been filed for this unsigned inventor			
Given Name	Venkatraghavan	Middle Name		Family Name	Vasudevan	Suffix e.g. Jr.	
Inventor's Signature	<i>Venkatraghavan Vasudevan</i>				Date	12-12-01	
Residence: City	Indianapolis	State	IN	Country	USA	Citizenship	USA
Address	1016 Saratoga Circle						
Post Office Address	SAME AS ABOVE						
City	Indianapolis	State	IN	Zip	46280	Country	USA

Name of Additional Joint Inventor, if any:				<input type="checkbox"/> A Petition has been filed for this unsigned inventor			
Given Name	Mark	Middle Name	James	Family Name	Zweifel	Suffix e.g. Jr.	
Inventor's Signature	<i>Mark James Zweifel</i>				Date	11-28-01	
Residence: City	Mooresville	State	IN	Country	USA	Citizenship	USA
Post Office Address	1840 Centenary Road						
Post Office Address	SAME AS ABOVE						
City	Mooresville	State	IN	Zip	46158	Country	USA

Name of Additional Joint Inventor, if any:				<input type="checkbox"/> A Petition has been filed for this unsigned inventor			
Given Name		Middle Name		Family Name		Suffix e.g. Jr.	
Inventor's Signature					Date		
Residence: City		State		Country		Citizenship	
Post Office Address							
Post Office Address	SAME AS ABOVE						
City		State		Zip		Country	

Name of Additional Joint Inventor, if any:				<input type="checkbox"/> A Petition has been filed for this unsigned inventor			
Given Name		Middle Name		Family Name		Suffix e.g. Jr.	
Inventor's Signature					Date		
Residence: City		State		Country		Citizenship	
Post Office Address							
Post Office Address	SAME AS ABOVE						
City		State		Zip		Country	

Please type a plus sign (+) inside this box ☐

PTO/SB/01 (8-96) (MODIFIED)

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DECLARATION FOR UTILITY OR DESIGN PATENT APPLICATION

☒ Declaration Submitted with Initial Filing
☐ Declaration Submitted after Initial Filing

Attorney Docket Number	X-11260
First Named Inventor	Matthew David Belvo
COMPLETE IF KNOWN	
Application Number	
Filing Date	
Group Art Unit	
Examiner Name	

As a below named inventor, I hereby declare that:

My residence, post office address, and citizenship are as stated below next to my name.

I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled:

PSEUDOMYCIN N-ACYL SIDE-CHAIN ANALOGS

the specification of which
☐ is attached hereto
OR

☒ was filed on
(MM/DD/YYYY)

06 June 2000 as United States Application Number or PCT International

Application Number PCT/US00/15017 and was amended on (MM/DD/YYYY) (if applicable).

I hereby state that I have reviewed and understand the contents of the above-identified specification, including the claims, as amended by any amendment specifically referred to above.

I acknowledge the duty to disclose information which is material to patentability as defined in Title 37 Code of Federal Regulations, § 1.56.

I hereby claim foreign priority benefits under Title 35, United States Code § 119(a)-(d) or § 365(b) of any foreign application(s) for patent or inventor's certificate, or § 365(a) of any PCT international application which designated at least one country other than the United States of America, listed below and have also identified below, by checking the box, any foreign application for patent or inventor's certificate, or of any PCT international application having a filing date before that of the application on which priority is claimed.

Priority Foreign Application Number(s)	Country	Foreign Filing Date (MM/DD/YYYY)	Priority Not Claimed	Certified Copy Attached	
				YES	NO
			<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
			<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
			<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
			<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
			<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
			<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>

☐ Additional foreign application numbers are listed on a supplemental priority sheet attached hereto:

I hereby claim the benefit under Title 35, United States Code § 119(e) of any United States provisional application(s) listed below.

Application Number(s)	Filing Date (MM/DD/YYYY)	<input type="checkbox"/> Additional provisional application numbers are listed on a supplemental priority sheet attached hereto.
60/143,989	15 July 1999	

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PTO/SB/01 (8-96) (MODIFIED)

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Patent and Trademark Office: U.S. DEPARTMENT OF COMMERCE

DECLARATION

I hereby claim the benefit under Title 35, United States Code §120 of any United States application(s), or § 365(c) of any PCT international application designating the United States of America, listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States or PCT international application in the manner provided by the first paragraph of Title 35, United States Code § 112, I acknowledge the duty to disclose information which is material to patentability as defined in Title 37, Code of Federal Regulations § 1.56 which became available between the filing date of the prior application and the national or PCT international filing date of this application.

U.S. Parent Application Number	PCT Parent Number	Parent Filing Date (MM/DD/YYYY)	Parent Patent Number (if applicable)

☐ Additional U.S. or PCT international application numbers are listed on a supplemental priority sheet attached hereto.

As a named inventor, I hereby appoint the following registered practitioner(s) to prosecute this application and to transact all business in the Patent and Trademark Office connected therewith:

Attorney Name	Reg. No.
Arvie J. Anderson	45,253
Lynn D. Apelgren	45,341
Robert A. Armitage	27,417
Brian P. Barrett	39,597
Michael T. Bates	34,121
Roger S. Benjamin	27,025
Gary M. Birch	48,881
William R. Boudreaux	35,196
Steven P. Cantinder	36,467
Paul R. Cantrell	36,470
Charles E. Cohen	34,565
Donald L. Corniglio	30,741
Gregory A. Cox	47,504
Paula K. Davis	47,517
Elizabeth A. Dawalt	44,646
John C. Demeter	30,167
Manisha A. Desai	43,585
Joanne Longo Feeney	35,134
Paul J. Gayto	36,808
Francis O. Gnah	44,712
Janet A. Gongola	48,436
Amy E. Hamilton	33,894
Frederick D. Hunter	26,915
Thomas E. Jackson	33,064
Charles Joyner	30,466
Gerald P. Keleher	43,707

Attorney Name	Reg. No.
James J. Kelley	41,288
Paul J. Kolvuniemi	31,533
Robert E. Lee	27,919
Kirby Lee	47,744
James P. Leeds	35,241
Nelsen L. Lentz	36,537
Douglas K. Norman	33,267
Arleen Palmberg	40,422
Thomas G. Plant	35,784
Edward Prein	37,212
Grant E. Reed	41,264
James J. Sales	33,773
Michael J. Sayles	32,295
Robert L. Sharp	45,606
David M. Stemmerick	40,187
Mark J. Stewart	43,936
Robert D. Titus	40,206
Robert C. Tucker	45,165
Tina M. Tucker	47,145
MaChari Vomdran-Jones	36,711
Gilbert T. Voy	43,972
Thomas D. Webster	39,872
Lawrence T. Welch	29,487
Alexander Wilson	45,782
Dan L. Wood	48,613

☐ Additional registered practitioner(s) named on a supplemental sheet attached hereto.

Direct all correspondence to:

Name	ELI LILLY AND COMPANY				
Address	ATTN: TINA M. TUCKER				
Address	LILLY CORPORATE CENTER/DC1104				
City	INDIANAPOLIS	State	INDIANA	ZIP	46285
Country		Telephone	(317) 277-3537	Fax	(317) 276-3861

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Name of Sole or First Inventor:

☐ A Petition has been filed for this unsigned inventor

Given Name	Matthew	Middle Name	David	Family Name	Belvo	Suffix e.g. Jr.	
Inventor's Signature							Date
Residence: City	Greenfield	State	IN	Country	USA	Citizenship	USA
Address	24 Greenbrook Court						
Post Office Address	SAME AS ABOVE						
City	Greenfield	State	IN	Zip	46140	Country	USA

☒ Additional Inventors are being named on supplement sheet(s) attached hereto.

Please type a plus sign (+) inside this box

+

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Patent and Trademark Office: U.S. DEPARTMENT OF COMMERCE**DECLARATION**

Name of Additional Joint Inventor, if any:		<input type="checkbox"/> A Petition has been filed for this unsigned inventor					
Given Name	Shu	Middle Name	Hui	Family Name	Chen	Suffix e.g. Jr.	
Inventor's Signature						Date	
Residence: City	Carmel	State	IN	Country	USA	Citizenship	USA
Address	13256 Snow Owl Drive						
Post Office Address	SAME AS ABOVE						
City	Carmel	State	IN	Zip	46033	Country	USA

Name of Additional Joint Inventor, if any:		<input type="checkbox"/> A Petition has been filed for this unsigned inventor					
Given Name	Christopher	Middle Name	William	Family Name	Doecke	Suffix e.g. Jr.	
Inventor's Signature						Date	
Residence: City	Indianapolis	State	IN	Country	USA	Citizenship	USA
Post Office Address	7515 East 80 th Street						
Post Office Address	SAME AS ABOVE						
City	Indianapolis	State	IN	Zip	46256	Country	USA

Name of Additional Joint Inventor, if any:		<input type="checkbox"/> A Petition has been filed for this unsigned inventor					
Given Name	Sarah	Middle Name	Lynne	Family Name	Hellman	Suffix e.g. Jr.	
Inventor's Signature						Date	
Residence: City	Indianapolis	State	IN	Country	USA	Citizenship	USA
Post Office Address	7009 Ringtail Court						
Post Office Address	SAME AS ABOVE						
City	Indianapolis	State	IN	Zip	46254	Country	USA

Name of Additional Joint Inventor, if any:		<input type="checkbox"/> A Petition has been filed for this unsigned inventor					
Given Name	James	Middle Name	Andrew	Family Name	Jamison	Suffix e.g. Jr.	
Inventor's Signature						Date	
Residence: City	Indianapolis	State	IN	Country	USA	Citizenship	USA
Post Office Address	7009 Ringtail Court						
Post Office Address	SAME AS ABOVE						
City	Indianapolis	State	IN	Zip	46254	Country	USA

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DECLARATION

Name of Additional Joint Inventor, if any:		<input type="checkbox"/> A Petition has been filed for this unsigned inventor					
Given Name	Venkatraghavan	Middle Name		Family Name	Vasudevan	Suffix	e.g. Jr.
Inventor's Signature							Date
Residence: City	Indianapolis	State	IN	Country	USA	Citizenship	USA
Address	1016 Saratoga Circle						
Post Office Address	SAME AS ABOVE						
City	Indianapolis	State	IN	Zip	46280	Country	USA

Name of Additional Joint Inventor, if any:		<input type="checkbox"/> A Petition has been filed for this unsigned inventor					
Given Name	Mark	Middle Name	James	Family Name	Zweifel	Suffix	e.g. Jr.
Inventor's Signature							Date
Residence: City	Mooresville	State	IN	Country	USA	Citizenship	USA
Post Office Address	1840 Centenary Road						
Post Office Address	SAME AS ABOVE						
City	Mooresville	State	IN	Zip	46158	Country	USA

Name of Additional Joint Inventor, if any:		<input type="checkbox"/> A Petition has been filed for this unsigned inventor					
Given Name		Middle Name		Family Name		Suffix	e.g. Jr.
Inventor's Signature							Date
Residence: City		State		Country		Citizenship	
Post Office Address							
Post Office Address	SAME AS ABOVE						
City		State		Zip		Country	

Name of Additional Joint Inventor, if any:		<input type="checkbox"/> A Petition has been filed for this unsigned inventor					
Given Name		Middle Name		Family Name		Suffix	e.g. Jr.
Inventor's Signature							Date
Residence: City		State		Country		Citizenship	
Post Office Address							
Post Office Address	SAME AS ABOVE						
City		State		Zip		Country	